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CLINICAL AND EXPERIMENTAL

SODIUM THIOSULPHATE AND CALCIUM SALTS IN PREVENTION OF THE SEQUELAE OF ILLUMINATING GAS POISONING*

BY W. H. ZEIGLER,† PHAR.D., CHARLESTON, SOUTH CAROLINA

THIS report has to do with a study of the sequelae of acute illuminating gas poisoning together with an outline of a treatment for their prevention.

Poisoning from this gas may be divided into acute, chronic and relapsing types. The symptoms of acute poisoning are well known, but the sequelae have never been given the emphasis they should have. The tendency in cases of acute poisoning is to render first aid by removing the patient from the atmosphere of the gas, by using the pulmotor or some other form of artificial respiration, by administering oxygen or oxygen plus carbon dioxide by inhalation until the patient returns to consciousness, and then to feel that we have done our duty. To quote Henderson and Haggard,[1] who are authorities on illuminating gas poisoning: "Within a few hours after profound but not fatal poisoning from carbon monoxid no trace of the gas is found in the blood, and yet for days, months, or even for life, structural degenerations, usually either nervous or cardiac, may continue." They also give as their opinion that, "during the first hour about half the amount previously absorbed is eliminated" and suggest that "the post-gassing period of continued asphyxia is of critical importance in inducing structural degeneration and functional impairment."

Kurlander[2] sums up the pathologic changes as follows: "There are marked degenerative changes in the muscles, and, in most instances, small scattered hemorrhages and intense hyperemia of the organs. The most important nerve lesions are in the brain."

*Read in abstract before the Medical Society of South Carolina, January 27, 1926.
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The sequelae of acute poisoning from this gas are summed up by McConnell and Spiller[3] as follows: "Pneumonia, cardiac palpitation, localized hyperemia, gastrointestinal disturbances, transient glycosuria, cutaneous eruptions, localized edema and gangrene. In the nervous system, paralysis of the central or peripheral type. Persistant headache is complained of and mental changes take place, often only mild hallucinations but more commonly distinct confusional insanities. Relapsing carbon monoxid poisoning is a term used to indicate the condition in those who had apparently recovered from the initial effects of the poison only to develop, after a period of fair health, a grave type of symptoms leading to death."

Carbon monoxid is the toxic agent in illuminating gas although it is the opinion of Henderson and Haggard[1] that 25 per cent of its toxicity is due to some other substance. We have been taught that carbon monoxid combines with hemoglobin to form a strong combination which is very difficult to break up. The opinion of authorities today is that while it combines with the hemoglobin, the combination is easily broken up. If this is true, how can we explain the sequelae and the delayed symptoms? It has been suggested, as an explanation, that in carbon monoxid poisoning: "by imperfect elimination injurious metabolic substances accumulate and produce profound effects upon the cerebral vessels."

It was not the intention of the author of this investigation to seek for causes but to attempt to find some method of preventing the sequelae so often seen in this class of poisoning. With this idea in view, a series of experiments were planned in which the animals used were dogs. After a number of preliminary experiments with different percentages of the gas, an amount was found which would produce mental and other delayed symptoms in the animal.

EXPERIMENTS

Dogs were the animals used throughout the investigation. The animals were placed in a specially constructed chamber provided with a glass door through which they could be watched. City illuminating gas was delivered through a tube connected to a box. A very delicate gas meter was used to register accurately the amount of the gas necessary to give a 2 per cent atmosphere

PROTOCOL

Two per cent illuminating gas followed by the intravenous injection of 5 c.c. of a 2 per cent solution of sodium thiosulphate per kilo and the subcutaneous injection of 2 c.c. of a 1 per cent solution of calcium lactate per kilo.

Dog, 6.8 kg. July 23, 1925.	10:45 A.M.	2 per cent illuminating gas.
	10:50 A.M.	Discomfort with excessive salivation.
	10:55 A.M.	Struggles and whines, falls.
	11:00 A.M.	Down.
	11:02 A.M.	Vomits.
	11:10 A.M.	Convulsions, skin deep red.
	11:20 A.M.	Respiration shallow and rapid.
	11:31 A.M.	Removed from chamber.
	11:35 A.M.	5 c.c. 2 per cent solution per kg. of sodium thiosulphate given intravenously. Also 2 c.c. 1 per cent calcium lactate per kg. injected subcutaneously.

	11:40 A.M.	Evidence of recovery.
	12:10 P.M.	Gets up and walks around. Staggers and seems confused.
	12:36 P.M.	2 c.c. 1 per cent calcium lactate per kg. subcutaneously.
	1:00 P.M.	Walks around.
July 24,		Fully recovered.
August 21,		Alive.

TABLE I

GAS 2 PER CENT, WITHOUT TREATMENT

NUMBER	IN CHAMBER	CONDITION ON REMOVING	
1	35 min.	Gasping	Died before treatment could be given
2	25 min.	Gasping	Died before treatment could be given
3	25 min.	Gasping	Died before treatment could be given
4	28 min.	Not breathing	Died before treatment could be given
5	30 min.	Breathing	Died before treatment could be given
6	40 min.	Gasping	Died before treatment could be given
7	38 min.	Gasping	Died before treatment could be given
8	25 min.	Gasping	Died before treatment could be given
9	30 min.	Not breathing	Died before treatment could be given
10	28 min.	Gasping	Died before treatment could be given
11	25 min.	In convulsion	Died before treatment could be given
12	30 min.	Gasping	Died before treatment could be given

in the chamber. The illuminating gas used contained about 25.2 per cent of carbon monoxid. One-tenth of one per cent of carbon monoxid will prove fatal in time, and 1 per cent will cause death in a few minutes. An atmosphere of 2 per cent illuminating gas was decided upon because of this and also due to the fact that after a number of experiments with varying amounts of the gas, it was found to be fatal in the majority of cases. Protocol will show how quickly the first symptoms were produced. These usually came in less than five minutes. The animals were allowed to remain in the chamber until the respiration had almost ceased. As seen by the records, this varied. The shortest time being twenty-seven minutes and the longest, fifty minutes. Table I will show, by the number of deaths which occurred before treatment could be given, how close the amount of gas used and the time of exposure approached the fatal dose.

After the proper amount of gas was determined, together with the time the animals were to be subjected to its influence, the next step was to find some substance which would chemically change or combine with the carbon monoxid of the illuminating gas. Since it is quite evident that "this very poisonous gas penetrates rapidly into the cells, the success of any treatment would depend upon the rapidity with which it could be distoxicated."

Meyer and Gottlieb[4] give as their opinion: "When the combination formed by the poison and the protoplasm, or more correctly the reacting constituent thereof, is reversible with difficulty or not at all (for instance, on account of its complete insolubility), it is quite clear that even an adequate antidote, which is able to combine with the poison, cannot reverse the toxic reaction. In such cases, however, it may be possible to repair the protoplasm by replacing such of its constituents as have combined with the toxic agent. This is actually what occurs in the antagonistic action of lime salts in oxalate poisoning. When, on the other hand, the toxic reaction is readily reversible, as for example in chloral

or chloroform poisoning, a substance which possesses an avidity for the toxic substance equal to or greater than that of the cell constituents can attract the toxic substance to itself and thus overcome the poisoning of the cell."

In the case reported by McConnell and Spiller,[3] there was a marked calcification of the capillaries of the brain, being more pronounced in the segments of the lenticular nucleus. They suggest that this condition is due to the calcium salts of the blood being thrown out of solution, caused by utilization of the proteids which held them in solution or suspension. Since sodium thiosulphate had been used with success in poisoning by lead tetraethyl, the nitrils, and cyanides, it was thought that perhaps this chemical would distoxicate or break up the combination of the carbon monoxid with the hemoglobin more rapidly.

Fig. 1.—Photograph of one of the dogs on the twenty-first day, showing delayed symptoms.

Calcium chloride was also injected subcutaneously at regular intervals with the hope of replacing this element and at the same time increasing the coagulability of the blood.

After a number of preliminary experiments in which measured amounts of sodium thiosulphate were injected intravenously and calcium chloride subcutaneously, the following procedure was found to be most effective. Immediately upon removing the animal from the chamber, artificial respiration was administered. If this was successful, 5 c.c. of a 2 per cent solution of sodium thiosulphate per kilo was injected into the vena saphena parva. At the same time, 2 c.c. of a 1 per cent solution of calcium chloride per kilo was injected subcutaneously. As seen by Table II, the recovery was not only more rapid, but, of the 14 dogs reported, only 1 developed mental symptoms and only 1 died. As seen by Table III, 12 dogs were subjected to the same percentage of gas over practically the same period of time without treatment. Two of the animals showed no mental symptoms; 2 recovered, 1 of these showing on the sixth day a weakness in the hind legs, and the other 8 after developing marked mental and other symptoms finally died.

TABLE II

GAS 2 PER CENT, WITH TREATMENT

NUMBER	IN CHAMBER	1ST SIGN OF RECOVERY	MENTAL SYMPTOMS	
1	33 min.	9 min.	None	Recovered
2	30 min.	6 min.	None	Recovered
3	31 min.	5 min.	None	Recovered
4	30 min.	8 min.	None	Recovered
5	36 min.	12 min.	None	Recovered
6	27 min.	8 min.	None	Recovered
7	43 min.	11 min.	None	Recovered
8	42 min.	3 min.	None	Recovered
9	41 min.	13 min.	None	Recovered
10	34 min.	5 min.	13th day drowsy	18th day died
11	28 min.	17 min.	None	Recovered
12	30 min.	10 min.	None	Recovered
13	33 min.	5 min.	None	Recovered
14	46 min.	14 min.	None	Recovered

TABLE III

GAS 2 PER CENT WITHOUT TREATMENT

NUMBER	IN CHAMBER	1ST SIGN OF RECOVERY	MENTAL SYMPTOMS	
1	46 min.	36 min.	26th day drowsy, irresponsive	28th day died
2	30 min.	15 min.	6th day paralysis of hind legs	8th day died
3	30 min.	10 min.	6th day weak in hind legs	Recovered
4	35 min.	23 min.	7th day weak and drowsy	10th day died
5	30 min.	30 min.	None	Recovered
6	40 min.	12 min.	16th day depressed with choreiform movements of muscles	20th day died
7	46 min.	19 min.	21st day weak and drowsy	22nd day died
8	39 min.	36 min.	3rd-8th day weak, nervous, disturbance of equilibrium	9th day died
9	50 min.	25 min.	2nd-25th day, depressed, irritable, disturbance of equilibrium	26th day died
10	30 min.	18 min.	8th day, had convulsion	37th day died
11	30 min.	12 min.	None	Recovered
12	31 min.	20 min.	40th day, drowsy	Recovered

Protocol gives an outline of the symptoms and treatment of the animals in this series of experiments.

The 38 dogs reported in the three tabulations were grouped according to results and not in sequence.

AN OUTLINE FOR TREATMENT IN HUMAN SUBJECTS

Up to the time of the preliminary report of this investigation, the author has not had the opportunity of demonstrating upon human subjects the treatment which was found effective for animals. I would suggest, however, as a result of these experiments, the following treatment: First remove the patient from the atmosphere of the illuminating gas. Administer artificial respiration if necessary and, as soon as possible, inject intravenously 5 c.c. of a 2 per cent solution of sodium thiosulphate for every 25 pounds of body weight. An individual weighing 150 pounds would require 30 c.c. of the solution. This amount would contain about 10 grains of the thiosulphate of soda. Kuhn and Reese[5] in a study of sodium thiosulphate in the treatment of metallic intoxication injected intravenously amounts of 15 grains over a period of fourteen days without any bad effects.

Sodium thiosulphate is oxidized in the body to sodium sulphate and a molecule of sulphur is liberated.

The next step in the treatment would be the subcutaneous injection of 5 c.c. of a 4 per cent solution of calcium lactate for each 25 pounds body weight. After consciousness has returned, calcium lactate should be given in a daily dose of 1 gram and continued for several days.

The following is a summary of a case which occurred in our city while this investigation was in progress and will serve to illustrate the sequelae of illuminating gas poisoning. The treatment consisted in the administration of chloral hydrate and sodium bromide.

CASE HISTORY

M. F., white, female, aged four years. On Sunday, January 9, patient was found in bathroom unconscious, asphyxiated with illuminating gas. Child was removed, resuscitated and brought to hospital. Regained normally and was sent home on Monday with apparently no ill effect. In the afternoon of the same day the child became very nervous and restless. The mother fearing convulsions returned the child to the hospital. General appearance of child was that of anxiety and restlessness. Physical examination was negative except for dilated pupils and hyperactive reflexes. Urinalysis negative. Temperature normal. W. B. C. 22,080. Polymorphonuclears 85.

Jan. 11. Child vomited. Rested well.
Jan. 12. Patient had several generalized convulsions in afternoon.
Jan. 13. Temperature 100°. Rather somnolent.
Jan. 14. No change.
Jan. 15. Urinalysis: Acetone ++++, others negative. Blood: 10,000. Polymorphonuclears 41. L. L. 44, etc. Tendency for child not to move upper extremities.
Jan. 16. Patient has paralysis of left upper extremities. Apparent inability to perceive objects although she does distinguish light. Pulse 72. Spinal fluid not under pressure. Analysis normal. X-ray shows no evidence of cranial injury.
Jan. 17. Patient had two convulsions in left side. Can move left hand.
Jan. 18. Has control of muscles. Retardation of muscular movement.
Jan. 19. Temperature normal, urinalysis normal. Improving.
Jan. 20. Improved.
Jan. 23. Patient discharged, apparently normal.

CONCLUSIONS

1. It is possible to produce in dogs the sequelae of carbon monoxid poisoning such as mental disturbances, general weakness, skin eruptions, etc.

2. It is possible to prevent these symptoms by the intravenous injection of sodium thiosulphate and the subcutaneous injection of calcium salts.

The author wishes to express his appreciation for the valuable assistance rendered by Dr. H. B. Holmes and Mr. E. C. Hood.

REFERENCES

[1]Henderson and Haggard: Jour. of Pharmacol. and Exper. Therap., 1921, v, 11.
[2]Kurlander, J. J.: Jour. Am. Med. Assn., 1924, lxxxiii, 271.
[3]McConnell, J. W., and Spiller, W. G.: Jour. Am. Med. Assn., 1912, lix, 2122.
[4]Meyer and Gottlieb: Pharmacol. Clin. Exper., 569.
[5]Kuhn, H. A., and Reese, H. H.: Jour. Am. Med. Assn., 1925, lxxxv, 1804.

THE EFFECT OF HYPODERMIC INSULIN ON THE FASTING BLOOD SUGAR IN NORMAL AND DIABETIC SUBJECTS IN RELATION TO PERCENTAGE NORMAL WEIGHT*

By Walter M. Bartlett, M.D., Boston, Mass.

IT has been noted by various observers that the effect of hypodermic injections of insulin as measured by the fall in blood sugar in mg. per 100 c.c. is largely dependent upon the level of the blood sugar at the time of injection.[1] In other words, it is common experience that insulin produces a more marked effect when the initial blood sugar level is high. In view of this fact it was thought that if insulin has a more specific blood-sugar-lowering effect in diabetic persons that it might offer a better diagnostic procedure than the usual glucose tolerance test. Consequently the blood sugar effect after hypodermic insulin has been studied in a series of cases including normal and diabetic subjects.

It was at first noted that the blood sugar effect was influenced by the relation of body weight to normal. This has offered the best method of classifying the cases.

TECHNIC

The patient's body weight and height were taken in the morning, the patient's fasting blood sugar determined and 10 units of U-20 Insulin-Lilly (Iletin) was given subcutaneously. Breakfast was omitted on the morning of the test, and subsequent blood sugar determinations were made at one hour and a half and three hours and a half after insulin. Blood sugar determinations were done by the Folin-Wu method.[2] Percentage normal weight was calculated from the tables of the Association of Life Insurance Directors and Actuarial Society of America.[3] The normal subjects referred to are in part healthy young adults and in part convalescent ward cases in whom we had no reason to expect any disturbance in carbohydrate metabolism.

DATA

Table I shows the effect of 10 units of insulin given hypodermically on the fasting blood sugar of ten normal subjects whose body weight is within 4 per cent of normal. The maximum fall in blood sugar occurred in each case three hours and a half after injection, and the average fall was 28 mg. per 100 c.c. of blood.

Table II shows the level of blood sugar before and after insulin in eight obese subjects whose body weight varies from 18 to 39 per cent above normal. Here the maximum fall in blood sugar occurred at the end of one hour and a half, and, although the fasting blood sugar was higher than in normal subjects of normal weight, the fall in blood sugar averaged 28 mg. as in Table I.

*From the Thorndike Memorial Laboratory of the Boston City Hospital, Boston, Mass. Received for publication, April 13, 1926.

TABLE I

EFFECT OF HYPODERMIC INSULIN IN NORMAL SUBJECTS

CASE NO.	INITIALS	AGE	WEIGHT	HEIGHT	% NORMAL WEIGHT	INSULIN	BLOOD SUGAR IN MG. PER 100 C.C.			MAXIMUM FALL	HR.
							Fasting	1.5 Hr.	3.5 Hr.		
		yr.	lb.	ft. in.		units	mg.	mg.	mg.	mg.	
1	N. S.	38	148	5′ 6″	±0	10	105	80	70	35	3.5
2	J. G.	59	178	5′ 11″	±0	10	98	65	59	39	3.5
3	W. P.	59	165	5′ 11″	−3	10	105	80	73	32	3.5
4	D. B.	24	154	5′ 10″	−1	10	86	77	75	11	3.5
5	S. B.	36	164	5′ 11″	−4	10	89	58	52	37	3.5
6	R. R.	39	168	5′ 11″	−3	10	74	69	65	9	3.5
7	S. K.	32	158	5′ 10″	−2	10	109	61	56	48	3.5
8	K. T.	28	154	5′ 10″	−2	10	86	75	72	14	3.5
9	D. V.	23	164	6′ 0″	±0	10	89	62	57	32	3.5
10	W. H.	54	167	5′ 10″	−2	10	105	78	70	35	3.5
										Average 28	

TABLE II

EFFECT OF HYPODERMIC INSULIN IN OBESE SUBJECTS

CASE NO.	INITIALS	AGE	WEIGHT	HEIGHT	% NORMAL WEIGHT	INSULIN	BLOOD SUGAR IN MG. PER 100 C.C.			MAXIMUM FALL	HR.
							Fasting	1.5 Hr.	3.5 Hr.		
		yr.	lb.	ft. in.		units	mg.	mg.	mg.	mg.	
1	M. M.	46	210	5′ 4″	+32	10	114	80	86	28	1.5
2	M. L.	56	216	5′ 2″	+36	10	112	78	88	34	1.5
3	H. W.	52	220	5′ 4″	+34	10	112	82	88	30	1.5
4	M. G.	58	198	5′ 8″	+18	10	108	82	86	26	1.5
5	H. R.	60	200	5′ 3″	+29	10	107	87	91	20	1.5
6	E. B.	38	215	5′ 2″	+39	10	125	89	93	36	1.5
7	G. W.	32	180	5′ 4″	+27	10	116	90	96	26	1.5
8	S. L.	39	196	5′ 5″	+28	10	106	81	88	25	1.5
										Average 28	

TABLE III

EFFECT OF HYPODERMIC INSULIN IN UNDERWEIGHT NORMAL SUBJECTS

CASE NO.	INITIALS	AGE	WEIGHT	HEIGHT	% NORMAL WEIGHT	INSULIN	BLOOD SUGAR IN MG. PER 100 C.C. Fasting	1.5 Hr.	3.5 Hr.	MAXIMUM FALL	HR.
		yr.	lb.	ft. in.		units	mg.	mg.	mg.	mg.	
1	T. T.	39	124	5′ 1″	− 6	10	110	46	60	64	1.5
2	J. C.	28	104	5′ 2″	−24	10	86	42	48	44	1.5
3	J. G.	67	110	5′ 4″	−32	10	88	46	52	42	1.5
4	G. C.	64	119	5′ 3″	−19	10	96	53	61	43	1.5
5	W. D.	55	148	5′ 10″	−17	10	107	61	82	46	1.5
6	J. W.	36	110	5′ 3″	−23	10	87	38	44	49	1.5
7	B. W.	56	139	5′ 9″	−21	10	107	56	59	51	1.5
8	T. W.	29	126	5′ 3″	− 5	10	112	50	64	62	1.5
9	A. D.	42	119	5′ 6″	−26	10	98	49	68	49	1.5
10	J. T.	49	112	5′ 3″	−26	10	86	44	62	42	1.5
										Average 59	

TABLE IV

EFFECT OF HYPODERMIC INSULIN IN DIABETIC SUBJECTS UNDER CONTROL

CASE NO.	INITIALS	AGE	WEIGHT	HEIGHT	% NORMAL WEIGHT	INSULIN	BLOOD SUGAR IN MG. PER 100 C.C. Fasting	1.5 Hr.	3.5 Hr.	MAXIMUM FALL	HR.
		yr.	lb.	ft. in.		units	mg.	mg.	mg.	mg.	
Patients above Normal Weight											
1	M. G.	62	180	5′ 10″	+ 5	10	160	128	99	51	3.5
2	C. R.	53	172	5′ 8″	+ 6	10	143	136	82	61	3.5
3	M. M.	48	168	5′ 3″	+17	10	126	120	90	36	3.5
4	V. S.	38	165	5′ 6″	+12	10	130	116	78	52	3.5
										Average 50	
Patients below Normal Weight											
5	G. R.	49	123	5′ 7″	−19	10	128	62	66	66	1.5
6	J. C.	8	47	4′ 1″	−15	10	120	42	55	78	1.5
7	R. B.	5	40	3′ 7″	− 5	10	122	46	61	76	1.5
8	W. R.	10	59	4′ 9″	−20	10	116	52	69	64	1.5
9	J. T.	36	117	5′ 6″	−25	10	132	62	84	70	1.5
										Average 70	

Table III shows the effect of 10 units of insulin given hypodermically on the fasting blood sugar of ten normal subjects whose body weight varied from 5 to 32 per cent below normal. In this series the maximum fall in blood sugar occurred after one hour and a half and the average extent of the fall was 59 mg. per 100 c.c. Nearly all of these cases developed premonitory signs of hypoglycemia including faintness, dizziness and perspiration. It was noted that in each case, however, these symptoms were but transitory and disappeared within a short time, although no antidote was given.

Table IV shows the effect of 10 units of insulin on the fasting blood sugar of diabetic subjects whose body weight was either above or below normal. In four cases above normal weight the maximum fall in blood sugar averaged 50 mg. in three and one-half hours. In five cases below normal weight the maximum fall in blood sugar averaged 70 mg. in one hour and a half. The underweight cases developed hypoglycemia; the overweight cases did not.

It is apparent from the foregoing tables that underweight subjects whether diabetic or normal are more sensitive to the blood-sugar-lowering effect of insulin. The fall in blood sugar following insulin in underweight subjects is not only greater in extent but occurs more rapidly than in normal subjects of normal weight. Obese diabetics are less sensitive to insulin than underweight diabetics and the blood sugar effect, although greater than in normal subjects of normal or above normal weight, is less than the effect in underweight normal persons but occurs more slowly than in obese normal persons. Obese normal persons and normal subjects of normal weight respond to the same extent to insulin, but its action is more rapid in the obese normal subject. The least effect of insulin occurs in the obese normal subject because the fasting blood sugar level is usually slightly higher than in normal subjects of normal or below normal weight, and yet the extent of the maximum fall after insulin is the same as in the normal subject of normal weight occurring, however, more rapidly. Since the blood sugar effect in underweight normal subjects simulates the effect in underweight diabetics so closely, the blood sugar curve following insulin cannot be used as a diagnostic test.

SUMMARY

1. Blood sugar curves following hypodermic insulin are reported in 37 cases.

2. Underweight normal subjects and underweight diabetic subjects were found most sensitive to the blood-sugar-lowering effect of insulin.

3. Obese normal subjects are the least sensitive to insulin.

4. The effect of insulin occurs most slowly in obese diabetics and normal subjects of normal weight.

5. The blood sugar curve following insulin is not specific enough in the diabetic patient to be used as a diagnostic test.

REFERENCES

[1]Fletcher, A. A., and Campbell, W. R.: The Blood Sugar Following Insulin Administration and the Symptom Complex-Hypoglycemia, Jour. Med. Research, 1922, ii, 639.

[2]Folin, O.: Laboratory Manual of Biological Chemistry, New York, 1923, D. Appleton & Co., 253.

[3]Association of Life Insurance Directors and Actuarial Society of America, New York, 1912, 38-67.

EPIDEMIC LYMPHADENITIS WITH FORMATION OF ABSCESS IN GUINEA PIGS DUE TO INFECTION WITH HEMOLYTIC STREPTOCOCCUS*

By John G. Hardenbergh, V.M.D., Rochester, Minn.

IN JANUARY, 1925, my attention was called to a guinea pig included in a group of normal animals that had been selected for diagnostic inoculations. This animal had a large, thin-walled abscess, 2.5 cm. in diameter, beneath the skin on the ventral part of the neck, containing thick, yellowish-white pus. A pure culture of a markedly hemolytic short-chained streptococcus was obtained in glucose-brain-broth and on blood-agar.

A survey of the normal guinea pigs held in stock revealed thirty-four animals out of about 750 with evidence of abscess similar to that in the first guinea pig. Thirty-one of these were females and three were males. Some of the animals had multiple swellings; a total of forty-three abscesses were recognized by palpation. The abscesses, which later were found to involve the lymphatics only, were distributed as follows: twenty-four in the cervical region; eleven in the submaxillary region; four in the precrural region, two in the parotid; and two in the inguinal region. Eight abscesses, all in females, were open and discharging. Frequent examinations of the supposedly normal guinea pigs from the stock were made for the next few weeks, and twenty-four more cases discovered, twenty-one in females and three in males. In all, fifty-eight spontaneous cases were examined between January 20, 1925, and February 21, 1925; fifty-one of these yielded pure cultures of hemolytic streptococci as found in the primary cases. The infection apparently ceased sometime in that month, and no more cases were seen for about a year.

On February 22, 1926, a new case apparently identical with the others was found under the same circumstances as the original one; each precrural region presented a round swelling about 1 cm. in diameter. The guinea pig was killed and examined; the precrural lymph nodes were found to be abscessed, and contained the characteristic thick, yellowish pus. Bacteriologic study again yielded a pure strain of hemolytic short-chained streptococci.

A survey of the stock guinea pigs revealed that a second enzoötic of the infection was present at almost exactly the same time of year as the first one. Thus far seven affected animals have been found in this second outbreak.

Table I gives data relative to the fifty-eight guinea pigs examined during the first outbreak, and Table II data of those examined to date in the second outbreak. Animals were killed with ether and the usual precautions observed in making cultures. A small amount of pus was drawn into a sterile pipette and transferred to a tube of glucose-brain-broth. After incubation for from eighteen

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TABLE I

DATA OF 1925 EPIDEMIC

DATE	GUINEA PIG	LYMPH NODES INVOLVED	ABSCESSES		RESULTS OF CULTURES	
			Number	Size, cm.	Glucose-brain-broth (primary culture)	Blood-agar (primary culture)
1-20	306	Cervical	1	3.0	Streptococci	Hemolytic streptococci
1-23	307	Submaxillary	1	2.5	Streptococci	Hemolytic streptococci
1-27	308	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
1-27	309	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
1-27	310	Submaxillary	1	1.5	Streptococci	Hemolytic streptococci
2- 3	311	Submaxillary	2	1.0	Streptococci	Hemolytic streptococci
2- 3	312	Inguinal	1	2.0	Streptococci	Hemolytic streptococci
2- 3	313	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2- 5	326	Right precrural	1	0.5		
		and submaxillary	2	0.5	Streptococci	Hemolytic streptococci
2- 5	327	Left precrural	1	1.0		
		and submaxillary	2	1.0	Streptococci	Hemolytic streptococci
2- 5	328	Submaxillary	4	0.5	Streptococci	Hemolytic streptococci
2- 5	329	Submaxillary	1	2.0	Streptococci	Hemolytic streptococci
2- 5	330	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2- 5	331	Inguinal	1	2.0	Streptococci and Bacillus coli	Hemolytic streptococci and *Bacillus coli*
2- 5	332	Parotid	1	0.5	Streptococci	Hemolytic streptococci
2- 5	333	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2- 6	334	Cervical	1	1.5	Streptococci	Hemolytic streptococci
2- 6	335	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2- 6	336	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2- 6	337	Parotid	1	1.5	Streptococci	Hemolytic streptococci
2- 6	338	Submaxillary and	1	0.5		
		each precrural	2	0.5	Streptococci	Hemolytic streptococci
2- 6	339	Submaxillary	1	1.5	Streptococci and *Bacillus subtilis*	Not cultured
2- 6	340	Right precrural	1	0.5		
		and submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2- 6	341	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2- 6	342	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2- 6	343	Right precrural	1	0.5		
		and submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2- 6	344	Cervical	1	1.5	Streptococci	Hemolytic streptococci
2- 6	345	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2- 9	358	Submaxillary	2	0.5	Streptococci	Hemolytic streptococci
2- 9	359	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2- 9	360	Cervical	2	1.0	Streptococci	Hemolytic streptococci
2- 9	361	Cervical	3	1.0	Streptococci	Hemolytic streptococci
2- 9	362	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2- 9	363	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2-11	372	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2-11	373	Submaxillary	2	0.3	Streptococci	Hemolytic streptococci
2-11	374	Submaxillary	2	1.5	Streptococci	Hemolytic streptococci
2-11	375	Submaxillary	1	2.0	Streptococci	Hemolytic streptococci
2-11	376	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2-11	377	Cervical (?)	?	?	No growth (node only inflamed)	
2-11	378	Cervical and	1	1.0		
		right precrural	1	0.5	Streptococci	Hemolytic streptococci
2-11	379	Right precrural	1	1.5	Streptococci	Hemolytic streptococci
2-11	380	Right precrural	1	0.5	Streptococci	Hemolytic streptococci
2-11	381	Submaxillary and	1	0.5	Streptococci	Hemolytic streptococci
		left precrural	1	0.5	Streptococci	Hemolytic streptococci
2-11	382	Submaxillary	3	1.5	Streptococci	Hemolytic streptococci
2-11	383	Left precrural	1	1.0	Streptococci	Hemolytic streptococci
2-18	393	Left precrural	1	1.5	Streptococci	Hemolytic streptococci
2-18	394	Submaxillary	1	1.0	Streptococci	Hemolytic streptococci
2-21	395	Submaxillary	1	0.5	No growth	
2-21	396	Left precrural	1	1.0	Streptococci	Hemolytic streptococci

TABLE I—CONT'D

DATE	GUINEA PIG	LYMPH NODES INVOLVED	ABSCESSES		RESULTS OF CULTURES	
			Number	Size, cm.	Glucose-brain-broth (primary culture)	Blood-agar (primary culture)
2–21	397	Cervical	1	1.5	Streptococci	Hemolytic streptococci
2–21	398	Left precrural	1	1.0	Streptococci	Hemolytic streptococci
2–21	399	Submaxillary	1			
		Cervical	1	1.0	Streptococci	Hemolytic streptococci
2–21	400	Cervical	1	0.5	Streptococci	Hemolytic streptococci
2–21	601	Cervical	1	1.0	Streptococci	Hemolytic streptococci
2–21	602	Submaxillary	2	0.5	No growth	
2–14	314*	Cervical	2	1.0	Streptococci and *Bacillus coli*	Hemolytic streptococci and *Bacillus coli*
		Precrural	1			
2–14	317*	Submaxillary	4	2.0	Streptococci	Hemolytic streptococci

*Died.

TABLE II

DATA OF 1926 EPIDEMIC

DATE	GUINEA PIG	LYMPH NODES INVOLVED	ABSCESSES		RESULTS OF CULTURES	
			Number	Size, cm.	Glucose-brain-broth (primary culture)	Blood-agar (primary culture)
2–22	700	Precrural	2	1.0	Streptococci	Hemolytic streptococci
2–23	701	Submaxillary	1	1.5	Streptococci	Hemolytic streptococci
2–23	702	Submaxillary	1	0.5	Streptococci	Hemolytic streptococci
2–23	703	Cervical	2	2.0	Streptococci	Hemolytic streptococci
2–23	704	Cervical	1	0.5	Streptococci	Hemolytic streptococci
2–23	705	Cervical	2	0.5	Streptococci	Hemolytic streptococci
2–23	706	Cervical	1	2.0	Streptococci	Hemolytic streptococci

to twenty-four hours, the resulting growth was examined and plated on blood-agar in order to observe hemolysis and to determine the purity of the culture.

A total of eighty-nine abscesses were found in the fifty-eight guinea pigs in the 1925 series, of which forty-five involved the submaxillary lymph nodes, twenty-six the cervical nodes, fourteen the precrural, two the parotid, and two the inguinal.

Pure cultures of hemolytic streptococci were obtained from the lesions of fifty-one of the fifty-eight guinea pigs; no growth was obtained in three; and in four, mixed cultures showing association or contamination with *Bacillus coli* twice, *Bacillus subtilis*, once, and *Staphylococcus albus* once.

SYMPTOMS AND LESIONS OF SPONTANEOUS CASES

The disease is symptomless except for the swelling, which may be visible or recognized only by palpation. The general health of the animals, with one or two exceptions, has not been impaired to any extent. The process is essentially one of the formation of "cold" abscesses involving the lymph nodes without evidence of reaction in the surrounding tissues except for the formation of a rather indefinite limiting membrane. Approximately half of the animals show involvement of the submaxillary lymph nodes; in one-fourth the precrural nodes are affected, and in the remaining fourth the lesions are divided among the cervical, parotid, and inguinal regions. The abscesses in the submaxillary and cervical regions may be single or multiple, varying from 3 mm. to 2.5 cm. in diameter. Multiple abscesses are seldom encountered in the other sites.

The pus contained in the abscesses is always thick, homogeneous, non-odorous and yellowish-white; apparently it never becomes cheesy or calcareous.

Stained smears show varying numbers of gram-positive streptococci, leucocytes, and a large amount of cellular débris.

The swellings are apparently not painful and may rupture spontaneously, after which the animal usually recovers slowly and uneventfully if good drainage is maintained for a sufficient period. Surgical drainage also leads to recovery.

The internal organs have not been found to be affected in cases of natural infection. The blood picture is variable and shows no significant change.

ETIOLOGY

It has been found by experiments that will be detailed later that the streptococci isolated from the abscesses are the exciting factor in the disease. It is difficult, however, to reproduce the chronicity of the natural infection because of the virulence of the organisms after transference to experimental animals. A sufficient number of chronic cases have followed artificial inoculation, however, to satisfy requirements for demonstrating the specificity of the isolated streptococci. The source and mode of the natural infection are undetermined.

MORPHOLOGIC AND CULTURAL CHARACTERISTICS

The streptococci when first isolated are uniformly gram-positive and acid-producing; in glucose-brain-broth or other suitable broth mediums, the chains formed are not long, usually consisting of from twelve to twenty organisms; single organisms and pairs are also seen; occasional long chained strains are encountered; the organisms are arranged as beads in the chains or as diplostreptococci; most of the strains present a flocculent growth with a marked tendency for the organisms to clump at the bottom of the tube in broth mediums, but an occasional strain will present diffuse clouding which has a tendency to persist. On blood-agar the separate colonies form wide areas of hemolysis; the colonies themselves are very small, delicate and only slightly raised. Only slight growth occurs on agar; gelatin is not liquefied. Litmus milk is made acid in twelve hours or less and the litmus is reduced; coagulation does not take place.

The effect of various strains on carbohydrates is quite constant. Acid is produced in dextrose, maltose, saccharose, salicin, lactose, and galactose; dulcite, levulose, and usually raffinose are also made slightly acid. According to Holman's classification, the infecting streptococci would be *Streptococcus pyogenes*.

PATHOGENICITY

Freshly isolated cultures are pathogenic for white mice, white rats, guinea pigs and rabbits. Subcutaneous inoculation induces the formation of local or diffuse abscesses with subcutaneous edema, hemolysis and a condition frequently resembling phelgmonous cellulitis; death occurs in from one to seven days. Intraperitoneal inoculation causes diffuse, purulent peritonitis that is rapidly fatal. Intravenous inoculation causes generalized septicemia that is fatal before any localized areas of infection can be recognized. Some strains have proved pathogenic for cats and dogs on intraperitoneal or intravenous injection but the factor of virulence for these animals is not constant.

EXPERIMENTAL REPRODUCTION

Several reproduction experiments were carried on. In the first of these, pure cultures of three different strains of the infecting streptococci were used (Table III).

TABLE III

SUBCUTANEOUS, INTRAPERITONEAL, AND INTRAVENOUS INOCULATION OF ANIMALS WITH PURE CULTURES OF STREPTOCOCCI*

ANIMALS INJECTED	MATERIAL		RESULT OF INJECTION
	Strain	Injection c.c.	
Guinea pig 384	372	0.2 subcutaneous	Death 2–17–25. Serosanguineous infiltration of subcutaneous tissue along entire abdominal wall; diffuse phlegmon. Pure cultures of hemolytic streptococci from heart blood, peritoneal fluid and subcutaneous exudate
Guinea pig 385	372	0.1 intraperitoneal	Death 2–17–25. Generalized purulent peritonitis. Pure cultures of hemolytic streptococci from heart blood and peritoneal exudate
Rabbit 386	372	0.1 intravenous	Death 2–18–25. Infarction of myocardium, right ventricle. Pure cultures of hemolytic streptococci from heart blood and spleen
Guinea pig 387	374	0.5 subcutaneous	Abscess at point of injection, 2–20–25; discharging 2–27–25; healed 3–17–25; superficial inguinal nodes involved 3–10–25 (animal killed). Abscesses in superficial inguinal nodes 1.5 cm. in diameter. Pure cultures hemolytic streptococci from both nodes; culture of heart blood sterile
Guinea pig 388	374	0.2 intraperitoneal	Animal well several days; slight reaction at point of injection; signs of pneumonia tenth day; very poor condition 2–27–25; died 3–11. Right lobar pneumonia with adhesions. Gram-positive diplococci from heart blood; green-producing streptococci from lung.
Rabbit 389	374	0.2 intravenous	Death 2–17–25. Petechiae in myocardium. Pure culture of hemolytic streptococci from heart blood
Guinea pig 390	376	1.0 subcutaneous	Death 2–17–25. Serosanguineous infiltration of subcutaneous tissues along entire abdominal wall; abscess at point of injection. Pure cultures of hemolytic streptococci from heart blood and peritoneal fluid; hemolytic streptococci and *Staphylococcus albus* from subcutaneous fluid
Guinea pig 391	376	0.5 intraperitoneal	Animal remained well until 3–17–25; killed. No lesions found. Cultures sterile
Rabbit 392	376	0.5 intravenous	Death 2–17–25. Edematous pericarditis. Pure cultures of hemolytic streptococci from heart blood

*Each animal injected 2-13-25.

It will be noted that subcutaneous inoculation of the organism killed two of three guinea pigs in four days and reproduced the natural infection in one, that is, caused the formation of local abscesses, from all of which the streptococci were recovered in pure culture. The most striking feature of subcutaneous inoculation is the widespread inflammatory process that is set up. Intraperitoneal inoculation caused the death from peritonitis of one guinea pig in four days, was a possible factor in pneumonia which proved fatal in a second, and had no effect in a third. Intravenous inoculation into rabibts caused septicemia that was fatal in four or five days. Further inoculation experiments with various strains of the streptococci are detailed in Table IV.

Attempted Infection by Mouth.—February 23, 1925, guinea pigs 607, 608 and 609 were fed 1 c.c. of a forty-eight-hour culture of streptococci from three spontaneous cases, in guinea pigs 396, 399 and 400, respectively. Guinea pigs 607 and 609 remained well for one month and showed no evidence of infection when killed; cultures from blood of the heart and spleen were sterile. Guinea pig 608 died March 23, 1925; it had become thin during the last week and death was due to pneumonia. There was no evidence of abscess in any part of the body and all cultures were sterile.

TABLE IV

INOCULATION WITH VARIOUS STRAINS OF STREPTOCOCCI

DATE 1925	ANIMALS INJECTED	INJECTION MATERIAL		INJECTION GLUCOSE BRAIN-BROTH		RESULT OF INJECTION
		Source	Age of culture	Amount c.c.	Mode	
2–23	Guinea pig 603	Guinea pig 396. Spontaneous; left precrural node	2 days	0.5	Subcutaneous	Local inflammation and swelling 3–1–25. Large abscess at point of injection 3–17–25. (Animal killed.) Abscess contained characteristic pus. Pure culture of hemolytic streptococci from abscess. Culture of heart blood sterile
2–23	Guinea pig 604	Guinea pig 399. Spontaneous; submaxillary node	2 days	0.5	Intraperitoneal	Death 2–26–25. Generalized, adhesive peritonitis with large amount thick, purulent exudate on all peritoneal surfaces. Culture of peritoneal fluid and heart blood yielded pure cultures hemolytic streptococci
2–23	Rabbit 605	Guinea pig 400. Spontaneous; cervical node	2 days	1.0	Subcutaneous	Local swelling and abscess formation 2–27–25. Death 3–3. Large area necrosis and abscess formation; generalized purulent peritonitis. Pure cultures hemolytic streptococci from abscess, heart blood and peritoneal fluid
2–23	Rabbit 606	Guinea pig 601. Spontaneous cervical node	2 days	0.5	Intravenous	Death 2–26–25. Pericarditis; hemorrhagic epicarditis; generalized adhesive peritonitis with large quantities of purulent exudate on peritoneal surfaces; pleurisy. Pure cultures from peritoneal fluid, heart blood and pleural fluid
3– 5	Rabbit 610	Rabbit 606	6 days	0.1	Intravenous	Animal remained well
3– 5	Rabbit 611	Rabbit 606	6 days	0.2	Intraperitoneal	Death 3–8–25. Generalized peritonitis, serofibrinous pleurisy, pericarditis, and small abscess at point of entrance of needle. Pure cultures hemolytic streptococci from abscess, peritoneal fluid, pleural fluid and heart blood
3– 5	Guinea pig 612	Rabbit 606	6 days	0.5	Intraperitoneal	Animal remained well

TABLE IV—CONT'D

DATE 1925	ANIMALS INJECTED	INJECTION MATERIAL		INJECTION GLUCOSE BRAIN-BROTH		RESULT OF INJECTION
		Source	Age of culture	Amount c.c.	Mode	
3- 5	Guinea pig 613	Rabbit 606	6 days	1.0	Subcutaneous	Local swelling and abscess formation 3–12–25. Large abscess 2 cm. diameter 3–17. (Animal killed.) Abscess contained characteristic pus. Pure cultures of hemolytic streptococci from abscess; heart blood sterile
3–18	Rabbit 616	Guinea pig 368. Artificially infected; abscess	24 hours	0.5	Intravenous	Animal remained well
3–18	Guinea pig 617	Guinea pig 368. Artificially infected; abscess	24 hours	0.5	Subcutaneous	Local swelling and abscess formation 3–25–25. Death 3–30. Large abscess at point of injection; left precrural lymph node also involved. Pure cultures hemolytic streptococci from abscess, heart blood and node
3–18	Guinea pig 618	Guinea pig 368. Artificially infected; abscess	24 hours	0.5	Intraperitoneal	Death 3–22–25. Extensive subcutaneous inflammation and beginning abscess formation along abdominal wall. Generalized peritonitis. Pure cultures hemolytic streptococci from heart blood, spinal and peritoneal fluid
3–18	Rabbit 619	Guinea pig 368. Spleen	24 hours	0.5	Intravenous	Animal remained well
3–18	Guinea pig 620	Guinea pig 368. Spleen	24 hours	0.5	Subcutaneous	Death 3–24–25. Extensive subcutaneous abscess formation and necrosis. Pure culture hemolytic streptococci from subcutaneous tissue and heart blood
3–18	Guinea pig 621	Guinea pig 368. Spleen	24 hours	0.5	Intraperitoneal	Death 3–25–25. Subcutaneous serosanguineous exudate. Large inflammatory mass in right inguinal region with beginning abscess formation. Pure culture hemolytic streptococci from abscess and peritoneal fluid

TABLE V

RESULT OF EXPOSING HEALTHY ANIMALS TO NATURALLY INFECTED ANIMALS

GROUP	GUINEA PIG SEX	CONDITION AT BEGINNING OF EXPERIMENT, 2–5–25	RESULT OF EXPOSURE
1	314, F	Spontaneously infected. Two large abscesses in cervical region. Not discharging	Death 2–14–25 (Table I)
	315, F	Normal; no sign of infection	Animal exposed for nine days only. No evidence of infection after two months
	316, M	Normal; no sign of infection	Animal exposed for nine days only. No evidence of infection after two months
2	317, M	Spontaneously infected. Four abscesses in submaxillary region and one in left precrural. Not discharging	Death 2–12–25 (Table I)
	318, F	Normal; no sign of infection	Animal exposed for seven days only. No evidence of infection after two months
	319, M	Normal; no sign of infection	Animal exposed for seven days only. No evidence of infection after two months
3	320, F	Spontaneously infected. Small discharging abscess in submaxillary region	Abscess continued to drain until entirely healed 3–5–25
	321, F	Normal; no sign of infection	Animal exposed for one month. No evidence of infection after two months
	322, M	Normal; no sign of infection	Animal exposed for one month. No evidence of infection after two months
4	323, M	Spontaneously infected. Small discharging abscess in submaxillary region	Abscess continued to drain for twelve days and was then entirely healed
	324, F	Normal; no sign of infection	No evidence of infection after two months
	325, M	Normal; no sign of infection	No evidence of infection after two months

Attempted Infection by Way of the Conjunctiva.—March 18, 1925, one drop of twenty-four-hour cultures of streptococci isolated from guinea pigs 388, 603 and 613 was applied to the conjunctiva of guinea pigs 622, 623 and 624, respectively. Conjunctivitis did not develop in the inoculated eyes, and the animals remained well for one month. Necropsy at this time revealed no evidence of streptococcus infection and all cultures were sterile.

Attempted Infection by Contact.—An experiment was undertaken to determine whether or not infection would readily occur through contact of healthy animals with naturally infected ones (Table V). Two of the naturally infected guinea pigs, 314 and 317, died seven and nine days, respectively, after the experiment was begun. These are the only two spontaneously infected animals that have died under my observation, and it is not certain that the streptococcus infection itself was the only factor. The other two spontaneously infected animals in this experiment recovered entirely after their abscesses had drained for several weeks. None of the exposed uninfected animals gave any evidence of having acquired the infection after two months' obsrvation and were found to be normal at necropsy.

Experiments with Regard to Toxin and Filtrable Virus.—The object of this experiment was to determine whether any toxin or filtrable substance was elaborated by the infecting streptococci or was contained in the abscess material which would produce lesions similar to those in spontaneously infected animals. For this experiment, four filtrates and one control emulsion of the pus from an abscess were prepared.

Filtrate 1.—Two tubes of pancreatic digest broth were inoculated with glucose brain cultures of strains of hemolytic streptococci isolated from guinea pigs 307 and 308. These were incubated at 37° C. for four days; an excellent growth of streptococci occurred, and the broth culture was passed through a sterile Berkefeld filter, and its sterility was proved by an attempt to culture it. This was used to inject guinea pigs 346, 347, 348 and 349 (Table VI).

Filtrate 2.—This was prepared the same as Filtrate 1 except that the organisms used were strains picked from blood-agar cultures isolated from guinea pigs 309, 310 and 311. The filtrate from these cultures was sterile and used to inject guinea pigs 350, 351, 352 and 353 (Table VI).

Filtrate 3.—Glucose-brain-broth tubes were inoculated with strains from guinea pigs 307, 308, 309, 310 and 311. Sterile filtrates were prepared as before, and used to inject guinea pigs 354, 355, 356 and 357 (Table VI).

Filtrate 4.—Three cubic centimeters of pus were collected in a sterile manner from the abscesses of spontaneously infected guinea pigs 359 and 360. This pus was mixed with 15 c.c. of sterile sodium chloride solution, then filtered through a sterile Berkefeld filter and cultured to determine its sterility. It was injected into guinea pigs 364, 365, 366 and 367 (Table VI).

Pus emulsion.—The residue remaining on the sterile Berkefeld candle used in preparing Filtrate 4 was washed off with sterile sodium chloride solution and usd to inject guinea pigs 368, 369, 370 and 371 (Table VI).

Necropsy Notes on Guinea Pigs Injected with Pus Emulsion.—Guinea pig 371 died February 11, 1925. Diffuse abscesses had formed about the point of injection between the skin and abdominal wall, and general peritonitis had developed. Cultures of the peritoneal exudate and heart blood yielded pure cultures of hemolytic streptococci.

Guinea pig 369 died February 13, 1925. Abscesses had begun to form at the point of injection, and serosanguineous infiltration had taken place in the subcutaneous tissue along the entire left side of the abdominal and thoracic walls. Cultures of the subcutaneous exudate and heart blood yielded pure cultures of hemolytic streptococci.

Guinea pig 371 died February 14, 1925. General peritonitis, exudative and adhesive, had set in. Cultures of the peritoneal exudate and heart blood yielded pure cultures of hemolytic streptococci.

Guinea pig 368 developed an abscess (Table VI) which opened and drained until February 27, 1925, when it was almost entirely healed. The animal was killed March 17. Slight inflammation was noted at the point of injection, a small abscess 0.5 cm. in diameter behind the right scapula, and three abscesses in the spleen, each 1 cm. in diameter; both precrural lymph nodes were inflamed and swollen. Cultures from the postscapular abscess and both precrural lymph nodes yielded pure cultures of hemolytic streptococci; culture of the heart blood was negative.

These experiments indicate that no substance such as a toxin is elaborated, either by culture of the hemolytic streptococci in vitro or by the organism in vivo, that is virulent for guinea pigs. The infectious nature of the purulent exudate is noted, however, in the experiment with the last series of guinea pigs in which three succumbed and one developed lesions like those in cases of spontaneous infection.

TABLE VI

RESULTS OF INOCULATING GUINEA PIGS WITH VARIOUS FILTRATES AND PUS EMULSION

GUINEA PIG	INJECTION 2-9-25			RESULT
	Amount c.c.	Material	Mode	
346	0.2	Filtrate 1	Subcutaneous	Animal remained well
347	1.0	Filtrate 1	Subcutaneous	Animal remained well
348	0.1	Filtrate 1	Intraperitoneal	Animal remained well
349	0.5	Filtrate 1	Intraperitoneal	Animal remained well
350	0.2	Filtrate 2	Subcutaneous	Animal remained well
351	1.0	Filtrate 2	Subcutaneous	Animal remained well
352	0.1	Filtrate 2	Intraperitoneal	Animal remained well
353	1.0	Filtrate 2	Intraperitoneal	Animal remained well
354	1.0	Filtrate 3	Subcutaneous	Animal remained well
355	2.0	Filtrate 3	Subcutaneous	Animal remained well
356	0.2	Filtrate 3	Intraperitoneal	Animal remained well
357	1.0	Filtrate 3	Intraperitoneal	Animal remained well
364	0.5	Filtrate 4	Subcutaneous	Animal remained well
365	1.0	Filtrate 4	Subcutaneous	Animal remained well
366	0.2	Filtrate 4	Intraperitoneal	Animal remained well
367	0.5	Filtrate 4	Intraperitoneal	Animal remained well
368	0.2	Pus emulsion	Subcutaneous	2-11-25 abscess 2 cm. diameter at point of injection 2-14-25 abscess opened spontaneously and draining 2-27-25 nearly healed. See necropsy notes
369	0.5	Pus emulsion	Subcutaneous	2-11-25 swelling at point of injection 2-13-25 died. See necropsy notes
370	0.1	Pus emulsion	Intraperitoneal	2-14-25 animal died. See necropsy notes
371	0.2	Pus emulsion	Intraperitoneal	2-11-25 animal died. See necropsy notes

Pathogenicity of Old Cultures.—On April 10, 1925, guinea pigs 625, 627 and 629 and rabbits 626, 628 and 630 were injected intraperitoneally with 1 c.c. of old glucose-brain-broth cultures obtained from guinea pigs 326, 327 and rabbit 392, respectively; the first two of these cultures were from spontaneous cases (Table I) and the last was isolated from an artificially infected animal (Table III). These strains were sixty-four and fifty-two days old, respectively, at the time of this experiment and had been kept in glucose-brain-broth at room temperature. Strains 326 and 327 were still virulent for guinea pigs and rabbits; strain 392 was avirulent.

Studies relating to the serology, immunology and production of hemolysin by the infecting streptococcus in this disease are being made and may form the basis for a future paper.

DISCUSSION

The condition described here is not to be confused with pseudotuberculosis of guinea pigs noted by several writers and caused by an organism designated as *Streptococcus pseudotuberculosis rodentium (Corneybacterium rodentium)*, a bacillus with rounded ends, short, thick, and gram-negative, having a tendency to form long chains in tissue as well as in artificial mediums. The lesions of so-called pseudotuberculosis of guinea pigs are usually found in the liver, spleen and intestine, and may be caseated, in contradistinction to the lesions of streptococcic lymphadenitis.

One of the most striking features in this study was the innocuousness of natural infection as compared with artificial infection. Few spontaneously in-

fected animals die, as the lesions are prone to rupture eventually and then heal. The epidemic nature of the infection and its apparently seasonal occurrence are characteristic of some other streptococcal infections in man and animals. The prevalence of the infection in female guinea pigs as compared to males in the ratio of about 8 to 1 would suggest infection by way of the genitalia, but this would hardly account for the apparent predilection of the organisms for the superficial lymph nodes.

In attempting to find the source of the infection, a careful check was made of all new guinea pigs received and it was found that infected animals had been received from several widely separated points. The possibility of infection from laboratory animals used for injection with various cultures of streptococci from human sources is further discounted by the fact that the reserve guinea pigs are held in stock pens in a building removed from the laboratory and are never in contact with used laboratory animals until placed on experiment. The disease, then, must be considered a natural affection.

The invasive powers of the infecting streptococci in artificial infection are illustrated by the fact that peritonitis was caused in one animal by intravenous inoculation (Rabbit 606, Table IV) and in another by subcutaneous inoculation (Rabbit 605, Table V).

Finally, the infection somewhat resembles a disease of horses commonly known as strangles (adenitis equorum) due to *Streptococcus equi* (Schütz), and which is characterized by inflammation of the nasal mucosa and suppuration of the regional lymph nodes. The equine disease, however, is an acute process with marked general disturbance and a mortality of from 2 to 5 per cent.

SUMMARY

A description is given of an epidemic disease in guinea pigs which is characterized by lymphadenitis with formation of abscesses in the superficial lymph nodes. The cause of the disease is a hemolytic streptococcus of the *pyogenes* group. The natural infection is chronic and comparatively innocuous; artificial infection is usually acute and rapidly fatal. The disease presents a seasonal variation and occurs more frequently in females than in males. The source and mode of the natural infection are not established. The etiologic significance of the infecting organism has been established.

MALIGNANT TUMORS OF THE ADRENAL IN CHILDREN*

With Report of a Case

By P. A. Bendixen, M.D., and F. H. Lamb, M.D., Davenport, Iowa

MALIGNANT tumors of the suprarenal glands have been recognized for many years, but it was not until 1907 that Robert Hutchinson[1] "first drew attention to a definite clinical syndrome, occasionally met with in children, consisting of cases of sarcoma of one or the other suprarenal with metastases in bones of the skull."[2] He then reported ten cases in all, seven of which were published for the first time. In view of the unique character of the lesion, the peculiar metastases, the striking clinical symptoms, and their occurrence only in children, it is rather strange that the condition had not received more attention before that time.

By way of orientation, it is worth reviewing briefly the relationship between malignant adrenal tumors and the clinical manifestations of these tumors with reference especially to their embryologic origin. The adrenal gland is composed of a cortex and medulla, derived from two separate primary germ layers. The cortex is of mesoblastic origin while the medulla is of neuroectodermic origin, the latter layer giving rise also to the sympathetic nervous system.

Bulloch and Sequeira[2] in 1905 collected and presented evidence showing the relation of altered suprarenal function and the development of the genital system. They discussed twelve cases of malignant neoplasms of the adrenal cortex in children, with additional reports of five necropsies, showing hypergenitalism to be associated with simple hypertrophy of the cortex. In another series of cases they pointed out the association of under-developed genitalia with hypoplastic or aplastic cortices,[3] confirming the observations of Wiesel[4] and Karakascheff[5].

Guthrie and Emery[6] in 1907, called attention to another syndrome in which cortical neoplasms were associated with precocious obesity and muscular hypertrophy.

Hutchinson in 1907 reported the series of cases, previously referred to, characterized by medullary tumors of the adrenal with their unique metastases to the bones of the skull. Rolleston[7] compares these selective metastases with the well-recognized association of carcinoma of the thyroid and skeletal metastases. According to the observations of Frew[8], there are "two entirely different clinical syndromes" in this disease with "equally different pathologic features, according to which suprarenal, right or left, had been the site of the primary growth."

Carter[9] has recently emphasized the generalization that tumors of the suprarenal medulla do not cause sexual prematurity, but usually manifest them-

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selves first by metastases and resulting local symptoms and signs, as illustrated in the report of our case.

There are still other groups of adrenal tumors occurring in children, viz., the cases of congenital sarcoma of adrenal and liver in infants, and those cases of primary adrenal tumors not falling into any of the foregoing groups. Extensive references to these may be found in the contribution of Tileston and Wolbach "Tumors of the Adrenal Gland in Children."[10]

Since the clinical manifestations of adrenal tumors fall into groups which render possible a diagnosis during life, and since these syndromes in children differ from those of the adult renal tumors, the foregoing case is reported as an illustration of one of the most unique groups,—that of Hutchinson, with metastases to the skull (and in contrast to that group described by Pepper in which the metastases are entirely in the liver).

Fig. 1.—The right adrenal gland, gross appearance.

REPORT OF CASE

Social Status.—An American female child, five years of age, entered Mercy Hospital, Davenport, Iowa, March 12, 1924.

Principal Symptoms and Signs.—(1) Severe constant pain in the left frontal region of the head. (2) Pain in both legs as far as the ankles. (3) Loss of weight. (4) Anorexia. (5) Exophthalmos of the left eye. (6) Ecchymosis of the left lower eyelid.

Family History.—Father and mother are both well. The mother had a cystic tumor removed from the right ovary and an appendectomy in 1921. The father's mother died of uterine cancer at the age of sixty-four years. The patient had three brothers and two sisters all well.

Past Medical History.—Patient was a full-term baby with normal delivery. She was breast fed for nine months. She had influenza at two months and measles at two years of age. In December, 1923, and again in February, 1924, she fell out of bed, once receiving a slight wound above the left eye, which healed rapidly.

Present Illness.—Two and one-half months before, the mother noticed a slight protrusion of the left eye with discoloration under the lower lid. The exophthalmos became more and more prominent, and the ecchymosis gradually spread over the entire orbital region. One month after the onset a similar condition began in the right orbit.

The patient moaned during most of her waking hours, cried at times, and complained of severe pain in the head and left side of the face. At times she complained of pain in both thighs and legs. There has been a gradual loss of appetite and emaciation. During the last week of her life she became very apathetic, and on account of the bulging forehead, the

exophthalmos and ecchymosis, the emaciation, and the apparent hopelessness of the situation, she was truly an object of pity.

Physical Examination.—Ophthalmoscopic examination by Dr. Karl Vollmer, March 12, 1924: media, lens and vitreous clear; no inflammation of the eyeball itself; fundi showed a mild degree of papillitis with some tortuosity of retinal vessels, otherwise fundi were normal; child's vision was very defective. On account of the condition and age of the patient, it was difficult to determine the fields or amount of vision. She could see objects and persons

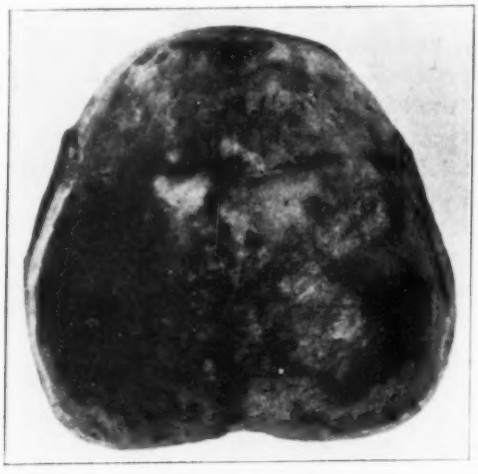

Fig. 2.—View of inside of skull cap.

Fig. 3.—View of outside of skull cap.

in the room. About one week later, March 17, the exophthalmos increased; the lids did not cover the sclera; the pupils were dilated and reflexes absent. The staring, due to the exophthalmos, was straight-forward at all times. The disc was pale, with slight cupping showing cribriform fascia, a picture of an atrophy following postorbital neuritis.

The heart and lung examinations were negative. Abdominal findings were negative. The presence of a new growth in the abdomen was suspected but could not be found.

The extremities were normal with the exception of emaciation. The external genitalia were normal.

The deep tendon reflexes were present; there was no patellar or ankle clonus. The skin reflexes were somewhat diminished.

Laboratory Findings.—Blood: R. B. C. 2,200,000, W. B. C. 5800, Hgb. 35 per cent. Moderate polychromasia and poikilocytosis. No pathologic leucocytes found. Wassermann negative.

Urine.—Sp. gr. 1.020. Chemical and microscopic examinations negative.

Spinal fluid.—Pressure 45 mm. Hg. Reduction of pressure to 10 mm. served to make the child more comfortable, but within 24 hours the pressure had returned to 45 mm. There were 4 lymphocytes per cu. mm., no globulin, no reduction, and a negative Wassermann.

Fig. 4.—Lateral view showing rarefaction of cranial bones.

Clinical Course.—The patient entered the hospital March 12, and died April 6, 1924. During this time the temperature ranged from 95.8° to 102° axillary. She was very restless and fretful, suffered a great deal of pain, and failed rapidly.

AUTOPSY REPORT

Body that of a female child, apparently about six years of age, very emaciated. Skin very pale, dry, and of fine texture. Hair of head is blond, about 12 cm. long. There is a prominent bulging of the scalp 8 cm. in diameter over the parietofrontal suture in the midline, also similar but smaller bulgings over the course of the parietooccipital sutures on each side and in the midline. The eyes are blue, decidedly protruded, the left slightly more than

the right; the sclerae are dull, pupils irregular, unequal and both dilated. Left upper and lower eyelid slightly everted. There are ecchymotic patches beneath each eye, extending down over the malar eminence, which, in contrast with the very pale skin, and blonde hair gives a grotesque appearance to the facies. The external nasal and auditory orifices, and buccal cavity are negative, mucosa very pale. There are no signs of trauma, wounds, anomalies or deformities.

Removal of scalp by an anterior and posterior flap method reveals dark red, rather firm thickenings of, or infiltrations beneath, the periosteum of the cranium. These are fairly well

Fig. 5.—Anterior-posterior of skull showing rarefaction of bones.

localized in irregular areas from 4-5 cm. to 10-12 cm. in diameter, following roughly the courses of the following suture lines—parietofrontal, parietooccipital, and parietotemporal.

Removal of the skull-cap shows grossly the same type of infiltrated areas on the inner aspect that were seen on the outer. The dura mater over the hemispheres is somewhat injected, the pia and arachnoid very pale.

The base of the skull shows many large irregular erosions of bone in the anterior and middle cranial fossae about equally extensive on both sides. Upon removing the roof of each orbital cavity it is found that the orbital contents are pushed to one side by a mass of dark

red firm tissue, resembling thickened portions of the skull-cap. Chiseling through the floors of both middle cranial fossae reveals this same dark red, firm infiltration extending down through sphenoid sinuses, partially filling the maxillary sinuses, and anteriorly into the ethmoid sinuses. The foramina in the floor of the middle fossae for the passage of the second, fifth, and sixth cranial nerves are eroded; in fact, the bone is almost replaced by infiltrated tissue. It is interesting to note that the dura over the base of the skull is intact, the erosive and infiltrative processes being limited to the bone itself, particularly around the foramina and along the suture lines.

The brain is very pale, vessels and venous sinuses containing but very little dark red, semiclotted blood. The size of the brain, the convolutions, and leptomeninges are normal. The ventricles contain a small amount of slightly turbid fluid, and are of normal size. On

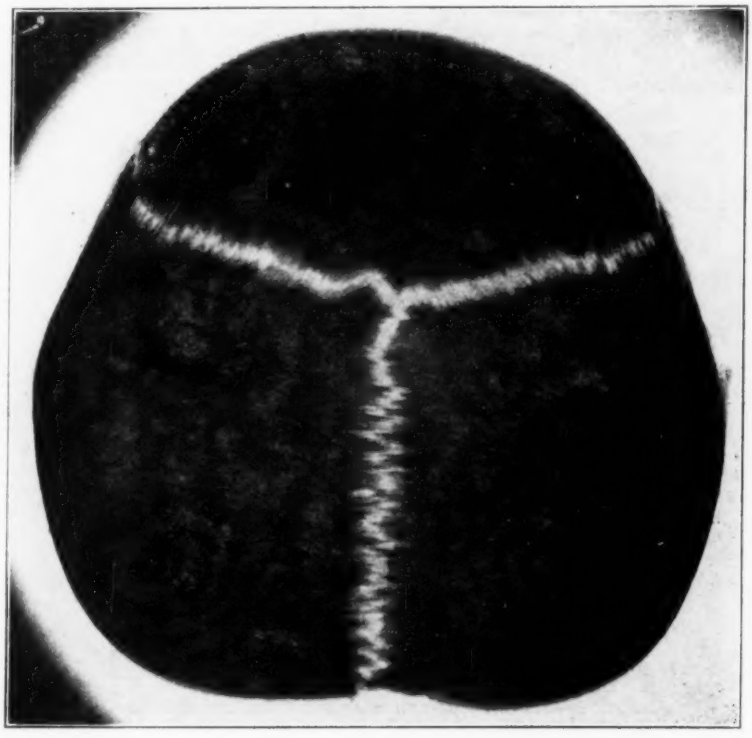

Fig. 6.—X-ray vault of skull showing rarefaction.

section, the brain substance is slightly softer than normal, and very pale. There are no signs of inflammation, hemorrhage, erosion, or infiltration, the dura having seemed to protect the brain completely from the erosion and infiltration so extensive in the bones of the cranium.

Permission for the autopsy having been limited to the head, and later granted for an exploration of the abdomen only, the thorax was not opened.

On median section of the abdomen, the subcutaneous fat is scanty, the muscles firm, and light red in color. There is no free gas or fluid in the abdominal cavity. The spleen is normal size, soft, and pale. The liver, normal in size and position, is of normal consistency and very pale. The capsule, for a patch, roughly oval about 5x8 cm. just over the right suprarenal glands, shows a marked thickening, averaging 20 mm., is white, very firm and resistant. The liver tissue beneath this area shows an area of apparently fatty degeneration, 0.5 to 1.0 cm. in depth, which gradually diminishes to blend with the more nearly normal appearing tissue. The stomach and intestinal canal are negative with the exception of the

very pale color. The pancreas seems normal. The left kidney is of normal size, capsule stripped easily, consistency normal; outlines between medullary rays and pyramids are well marked, the pelvis and ureter normal. The left suprarenal gland is normal in size and apparently so on section. The right kidney is similar to the left.

A search of the abdomen for evidence of metastases revealed none. The retroperitoneal lymph nodes are not enlarged, and are of normal color and consistency. Exploration of the large abdominal vessels was negative. There are no palpable lymph nodes, either in the abdomen or outside. The ribs, bones of the pelvis, and long bones are, so far as can be determined by palpation, negative for signs of metastases.

The right adrenal gland is about the size and shape of a large walnut. It is not adherent, lies 1 cm. above the upper pole of the right kidney, and is in contact with the inferior surface of the liver. This area is a white, firm, oval patch in the capsule as noted above. On section, the adrenal is firm; the cut surface is moist and contains a small amount of dark red blood. The cortex cannot be distinguished from the medulla. The dark red, granular appearance of the cut surface is a decided contrast to the typical yellow, fatty glistening cut surface of the hypernephroma.

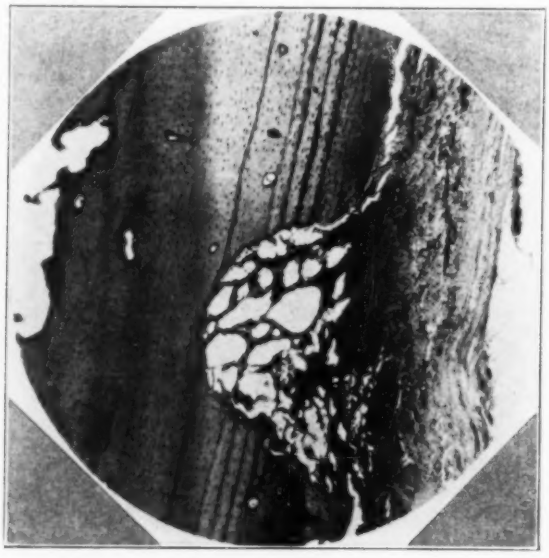

Fig. 7.—Microphotograph showing invasion of bone.

Examination of the tissue removed presented the histology of a typical neuroblastoma. The ground work of the tumor was composed of innumerable cells with scarcely perceptible cytoplasm and rounded or somewhat oval, richly chromatic nuclei lying in a homogeneous or finely fibrillated matrix staining pink with eosin. Mitotic figures were numerous. For the greater part, the cells were distributed diffusely and without any orderly arrangement, although occasionally they tended to form in parallel bundles, especially around the thicker and better formed blood vessels. Throughout the tumor at numerous intervals were rosettes made up of a ring of nuclei arranged radiately to a finely fibrillar or structureless substance staining pink with eosin. In some instances the rosettes were elongated and gave the appearance of alveoli. In some of the rosettes, the nuclei were arranged around structureless, jagged, bluish-staining bodies, suggesting calcium deposits. The tumor was hemorrhagic, and was frequently traversed by vascular channels, varying in size from that of the most delicate capillary to relatively large sinuses. The smaller of these channels were lined by a single layer of endothelium and evidently represented newly formed capillaries, but the larger sinuses were limited externally by compressed tumor cells. Free red corpuscles among the tumor cells occurred in great numbers.

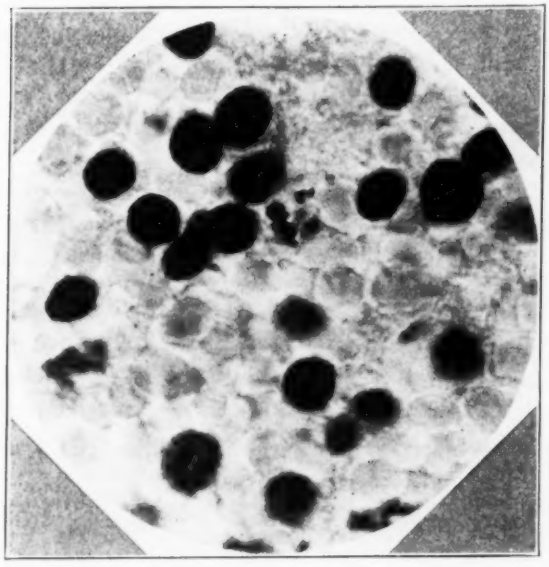

Fig. 8.—Microphotograph showing mitotic cell figures.

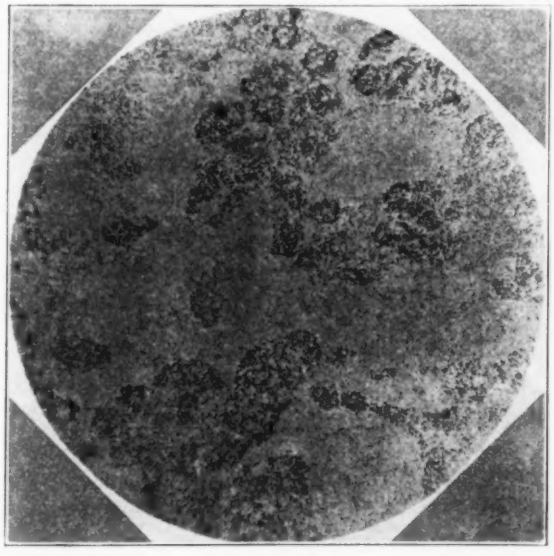

Fig. 9.—Microphotograph—low power of section of right adrenal gland.

SUMMARY

The neuroblastoma is a malignant tumor composed of undifferentiated nerve-cells or neuroblasts and springs most often from nests of such cells lying in the medulla of the adrenal capsule, but occasionally from identical cells existing in other localities. The tumor presents a characteristic histologic picture marked by the presence of delicate fibrils, supporting cells with scanty cytoplasm and richly chromatic, rounded nuclei, the cells being arranged diffusely, or in the form of rosettes, around tangled masses of fibrillated or homo-

geneous material, staining pinkish with eosin. In certain cases the fibrils are absent or poorly developed, rosettes cannot be seen; and the very cellular character of the tumor may, in these circumstances, lead to the diagnosis of sarcoma. Based on its cellular unit, the growth in question is most aptly provided for under the designation of neuroblastoma. Knowledge of the true derivation of these growths is of recent date. Virchow first mentioned the possibility. Dalton, Marchand and Kuster gave accurate histologic descriptions, but it was not until 1910 that Wright used the term, "Neuroblastoma," and since then Rich, Wahl, and others have firmly established the origin and histogenesis of these tumors.

CONCLUSION

1. Suprarenal medullary malignancy is not an altogether unusual occurrence.

2. In the majority of cases an orbital hemorrhage is the first sign observed, and it may occur before any tumor is palpable.

3. The orbit first involved is often on the same side as the primary tumor, although this case was an exception.

4. Diagnosis should not be difficult once the orbital hemorrhage has occurred; the disease is likely to be mistaken only for trauma, chloroma and scurvy.

5. Surgical interference can be of no avail, except as a palliative to drain a pyonephrosis or to meet other complications, as the metastases occur usually before a diagnosis can be made.

6. Metastases probably occur via the lymph stream.

7. The malignancy rarely metastasizes to the skin.

8. The medulla of the suprarenal gland being neurocctodermic in origin, these malignant tumors are similar in their histologic structure to malignant neoplasms of the sympathetic nervous system, and they are correctly designated as neuroblastoma.

REFERENCES

[1]Hutchinson, R.: Quart. Jour. Med., 1907, i, 33.
[2]Bullock, W., and Sequeira, J. H.: Tr. Path. Soc. London, 1905, lvi, 189.
[3]Hoag, L. A.: Am. Jour. Dis. Child., June, 1923, xxv, 6.
[4]Wiesel: Virchow's Arch. f. Path. Quart., 1904, clxxvi, 103.
[5]Karakascheff: Zieglers Beitr. z. Path. Quart., u. z. Allg. Path., 1904, xxx, 401.
[6]Guthrie and Emery: Tr. Clin. Soc. London, 1907, xl, 175.
[7]Rolleston, H. D.: Allbutt and Rolleston's System of Medicine, Section on "Ductless Glands," London, 1908, N. I., p. 429.
[8]Frew, R. S.: Quart. Jour. Med., 1911.
[9]Carter, W. E.: Am. Jour. Dis. Child., September, 1921, xxii, 224.
[10]Tileston, W., and Wolbach, S. B.: Am. Jour. Med. Sc., 1908, cxxxv, 871.

A NOTE ON CHLORETONE ANESTHESIA OF DOGS*

By W. T. Dawson, B.A. (Oxon.), Galveston, Texas

In 1917 Rowe[1] advised that chloretone should be administered intraperitoneally for dog anesthesia in one of two ways: (*a*) 1 c.c. per kg. of a 40 per cent solution of chloretone in 40 to 45 per cent alcohol (by volume), i.e., 0.4 gm. chloretone per kg., and (b) after preliminary morphine injection, one-half this amount of chloretone, or 0.2 gm. per kg. He advised against intraperitoneal injection in solution in warm oil, owing to "slow and uncertain" absorption. We have found that after preliminary morphine injection intraperitoneal administration of 1 c.c. per kg. of a solution of 10 gm. of chloretone in 70 c.c. of ether and 30 c.c. of liquid petrolatum, or 0.1 gm. of chloretone per kg., quickly produces a satisfactory anesthesia. We have also found that after preliminary morphine injection the intraperitoneal injection of 0.25 gm. per kg. in warm oil is, in a large percentage of dogs, followed by death from respiratory failure; and that in a number of other cases the anesthesia is very slow in developing.

Chloretone anesthesia was used extensively at the University of Texas during the first semester of 1925 to 1926. Over 100 dogs were prepared for student use in the laboratory of physiology by intraperitoneal injection of chloretone dissolved in warm liquid petrolatum or in cold petrolatum to which ether had been added. Morphine sulphate, about 2 to 5 mg. per kg., was first given subcutaneously; the weight of the dog, for this purpose, was merely estimated by a glance at the animal. When the dog had become quiet, usually in about twenty minutes, it was weighed, muzzled if necessary, laid on its back by two students of the group concerned, the hind legs pulled down, and injection of chloretone made through the lower half of the ventral abdominal wall, sufficiently high up to avoid the bladder. The needles used were always kept sharp. At first 0.25 gm. of chloretone per kg. was injected, dissolved in warm liquid petrolatum. It was found, however, that a number of the dogs—sometimes as high as one-third—showed respiratory failure before the experiments were well begun. Morphine, as is well known, depresses the respiratory center; and besides, the difficult solubility of chloretone, even when the oil was warmed, may have given rise to errors in dosage or to injection of oil at an unreasonably high temperature. The solvent power of the petrolatum was therefore increased by addition of ether. In order to avoid danger of overdose of anesthetic, when the ether was added to the solvent, the chloretone dose was reduced. The best results were obtained by the injection of 1 c.c. of the following anesthetic mixture per kg.: 30 c.c. of liquid petrolatum, 70 c.c. of ether, 10 gm. of chloretone. This corresponds to a dosage of about 0.1 gm. of chloretone per kg. After injection the dogs

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were left for fifteen minutes before the skin was actually incised; to give ether by inhalation during this period was evidently not advisable because of the large dose given intraperitoneally with the chloretone; the animals were, however, laid out on the board and the sites of operation cleaned and shaved. Additional inhaled ether during operation was sometimes necessary. Early deaths were infrequent.

In addition to the morphine, the animals are evidently under the influence, first, immediately after the injection, of 0.7 c.c. of ether per kg., promptly vaporized (boiling point, 34.6° C.) in the peritoneal sac; second, of ether plus chloretone, as the chloretone begins to be absorbed; and finally of chloretone alone as the ether is rapidly excreted through the lungs. The oil has, of course, no anesthetic property and is not required to bring the chloretone into solution, but it appears to make the mixture more easily taken up into the syringe without bubble formation, and also probably in some degree slows the absorption of chloretone after the injection.

Arterial blood pressure was sufficiently high to enable the performance of the usual student laboratory experiments upon factors influencing arterial blood pressure; these included even the effects of hemorrhage and infusion of citrated blood and were repeated several times upon the same animal. Observations upon salivary secretion were found perfectly practicable. The method was found satisfactory also in experiments in which the abdomen was opened, even within fifteen minutes of the intraperitoneal injection, e.g., kidney oncometer experiments, cannulation of the ureters or of the duct of the pancreas or of the bile duct, and preparation of secretin.

The anesthetic solution apparently keeps indefinitely in a well-stoppered bottle. Owing to its ready inflammability it should be kept away from flames during injection.

REFERENCE

[1]Rowe, L. W.: Trichlortertiarybutyl Alcohol Anesthesia, Jour. Pharmacol. and Exper. Therap., 1917, ix, 107.

AN INFECTION OF MAN PROBABLY DUE TO SALMONELLA SUIPESTIFER*

By Frederick W. Shaw, M.Sc., M.D., Richmond, Va.

IN 1922 Mackenzie[1] reported a case in which the causative organism described was found to conform in all its reactions to the Salmonella suipestifer. Since the available medical literature fails to show another case in which a bacillus of like reactions had been isolated from the blood of man, the writer believes the following report will be of interest.

CLINICAL

A man, aged fifty-five years, a railroad conductor, was admitted to Memorial Hospital, Richmond, Va., Nov. 18, 1925. He was taken ill on Nov. 15 but continued working until Nov. 17, when he became worse and called a physician. He had a cough and small amount of rusty sputum.

Patient was a well-developed, well-nourished, middle-aged white man. He seemed acutely ill and was semicomatose. Respirations fast. Pupils reacted. Heart apparently not enlarged; rate rapid; rhythm regular; sounds clear; no murmur. Lungs: percussion note resonant, except posteriorly at bases; breathing somewhat stertorous; there were, however, no gross changes; a shower of fine râles (inspiratory) was heard over right base, posteriorly; few squeaks scattered throughout. Abdomen soft and distended; no rigidity or apparent tenderness. No edema. Deep reflexes present in both extremities.

Diagnosis.—Acute lobar pneumonia (influenzal type). The temperature varied from 104.5° to 105° F.; the pulse rate was 125 on admission and increased until it was 140 per minute at 4 A.M. on Nov. 19; respirations were 40 per minute on admission and declined to 25 at 4 A.M. on Nov. 19.

Blood.—Wassermann test was negative; there were leucocytes 15,000 (neutrophyles 89 per cent, small lymphocytes 8 per cent, large mononuclears 3 per cent). The Widal test was positive in 1 to 20.

The patient died at 11 A.M. on Nov. 19, 1925.

Autopsy.—Bilateral chronic adhesive pleuritis; bilateral chronic interstitial pneumonia; multiple acute septic infarcts with beginning abscess formation in the left kidney; cloudy swelling of the liver, spleen and kidneys.

BACTERIOLOGY

The organism (S. No. 155) was isolated from the blood stream in pure culture. It was a nonlactose fermenting, nongelatin liquefying, gram-negative, motile, nonspore forming rod. Cultures on Russell's double sugar produced acid and gas but with alkaline slant; for this reason they resembled paratyphi and schotmuelleri in the above, but it did not agglutinate with the

*From the Department of Bacteriology, Medical College of Virginia.
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TABLE I

	MOTILITY	GELATIN	GRAM	MILK	LACTOSE	SACCHAROSE	DULCITE	MANNITE	DEXTROSE	MALTOSE	DEXTRIN	RAFFINOSE	ARABINOSE	TREHALOSE	INULIN	SORBITE	GALACTOSE	LEVULOSE	INOSITE	SALICIN	AMYGDALIN	GLYCERIN	RHAMNOSE	XYLOSE	LEAD ACETATE	VOGES.-PROS.	INDOL
S. paratyphi	+	–	–	A *Al*	O	O	AG	AG	AG	AG	O	O	AG	AG	O	AG	AG	AG	O	O	O	O A	AG	O	O	O	O
S. schotmuelleri	+	–	–	A *Al*	O	O	AG	AG	AG	AG	O	O	AG	AG	O	AG	AG	AG	AG	O	O	O	AG	AG	B	O	O
Paratyphoid "C"	+	–	–	A *Al*	O	O	AG	AG	AG	AG		O	AG		O	AG	AG	AG	O	O				AG			
S. enteritidis	+	–	–	A *Al*	O	O	AG	AG	AG	AG	O	O	AG	AG	O	AG	AG	AG	O	O	O	O	AG	AG	B		O
S. aertryeke (Newport)	+	–	–	A *Al*	O	O	AG	AG	AG	AG	O	O	AG		O	AG	AG	AG	O	O	O		AG	AG	O	O	O
S. aertryeke (Mutton)	+	–	–	A *Al*	O	O	AG	AG	AG	AG	O	O	AG		O	AG	AG	AG	AG	O	O		AG	AG	O	O	O
S. suipestifer	+	–	–	A *Al*	O	O	O[1]	AG	AG	AG	O	O	O	O	O	AG	AG	AG	O	O	O		AG	AG	O	O	O
Mackenzie	+	–	–	A *Al*	O	O	O[2]	AG	AG	AG		O	O		O	AG	AG	AG	O	O				AG			
#155 M. C. V.	+	–	–	A *Al*	O	O	O[2]	AG	AG	AG	O	O	O	O	O	AG	AG	AG	O	O	O	O	AG	AG	O	O	O

[1]Some strains produce acid and gas.
[2]Acid and gas on eighth day.
B = Blackened. *A* = Acid. *G* = Gas. *A, Al* = Acid then alkaline.

antisera for these organisms. This organism was then grown in fermentable substances, the results of which are shown in Table I.

An immune serum, which was prepared with this organism, agglutinated the organism in 1 to 25,600 dilution; but paratyphi and schotmuelleri did not agglutinate in 1 to 40. (Table II.)

TABLE II

AGGLUTININS

SERA	ORGANISMS			
	S. PARATYPHI	S. SCHOTTMUELLERI	S. SUIPESTIFER	S. #155
S. paratyphi	3200			40–
S. schottmuelleri		6400		40–
S. suipestifer			51200	51200
S. #155	40–	40–	25600	25600

TABLE III

ABSORPTION TESTS

SERA	ABSORBED WITH	ORGANISM AGGLUTINATED	TITER
S. #155	suipestifer	S. #155	480–
(original titer 25600)	S. #155	suipestifer	200–
S. suipestifer	suipestifer	S. #155	400–
(original titer 51200)	S. #155	suipestifer	400–

Two strains of Salmonella suipestifer agglutinated to full titer and absorbed the agglutinins for S. No. 155.

An immune serum was prepared with one of the suipestifer strains. The homologous organism agglutinated in 1 to 51,200 dilution. S. No. 155 agglutinated to full titer and absorbed the agglutinins for suipestifer. (Table III.)

Two rabbits were inoculated, intraperitoneally, with 0.25 c.c. of a twenty-four hour broth culture of S. No. 155. They died on the sixth day. Autopsy showed pale spots on the spleen, mottled liver, small hemorrhages in the lungs, congestion of the vessels in the small intestines, and hemorrhagic areas in the peritoneum.

DISCUSSION

It may be seen from Table I that this organism differs from Salmonella paratyphi in fermenting xylose and in not fermenting arabinose; from S. schottmuelleri and aertrycke (Mutton) in not fermenting arabinose and inosite, and from Para "C," aertrycke (Newport), and S. enteritidis in not fermenting arabinose. Also, it does not ferment dulcite until the eighth day, while the six organisms already mentioned ferment dulcite rapidly. Further, it does not ferment trehalose.

Mackenzie (loc. cit.) described the cultural characteristics of the organism isolated by him as growing readily on all ordinary laboratory media; nongelatin liquefier, and does not produce indol. In litmus milk it produced acid, which gave way after forty-eight hours to alkali and the milk remained strongly alkaline. He found that at the end of twenty-four hours' incubation there were acid and gas in the dextrose and maltose fermentation tubes, acid only in mannite, while lactose, saccharose and dulcite remained unchanged.

Arabinose and inosite remained permanently unchanged, but dulcite was later fermented with acid, and gas production began on the eighth day.

The organism under study (S. No. 155) produced acid and gas in dextrose, mannite, maltose, galactose, levulose, rhamnose and xylose in the first twenty-four hours but not in sorbite until the third day. This delay in sorbite was constant through a number of trials. Milk was acid in the first twenty-four hours and alkaline in forty-eight hours, remaining strongly alkaline. Indol and Voges-Proskauer were negative, and lead acetate was not blackened. Acid and gas were produced from dulcite, beginning on the eighth day.

Tenbroeck[2] studied a culture of S. icteroides that was received directly from Sanarelli. This organism agglutinated to the titer limit in anti-hog-cholera bacillus serum and absorbed the agglutinins from this serum. It failed to ferment dulcite and arabinose and did not produce H_2S. While conforming to suipestifer in fermentation and agglutination reactions, it differed from it in that it was not virulent for rabbits. He also reported that a culture in his collection labeled paratyphoid B Longcope, which culturally and serologically was a hog-cholera bacillus, would not kill rabbits. This organism, Tenbroeck thinks, came from a paratyphoid case reported by Longcope,[3] but he is not certain.

Cowern[4] reported several cases of persons suffering with the symptoms of so-called intestinal influenza. From the stools of these patients the Salmonella suipestifer was isolated at the London (Eng.) School of Tropical Medicine. No mention was made of the recovery of the bacillus from the blood stream. The case reported by Mackenzie (loc. cit.) was in Ireland, and the case under discussion in the present paper was in Richmond, Virginia; it seems probable that infections of man by this bacillus may be more numerous and widely distributed than is shown by the available medical literature.

CONCLUSION

The organism described conforms in its morphology, cultural characteristics, serologic reactions, and virulence to rabbits to that described by Mackenzie (loc. cit.) and to the strains of Salmonella suipestifer in my possession.

I wish to express my thanks to Dr. Charles Phillips, Professor of Pathology, Medical College of Virginia, who furnished me with the culture for identification, and to Mr. F. B. Morlok, Superintendent of the Hospital Division, Medical College of Virginia, for permission to make use of the clinical notes.

REFERENCES

[1]Mackenzie, J.: Jour. Roy. Army Med. Corps, 1922, xxxix, 51.
[2]Tenbroeck, C.: Jour. Exper. Med., 1920, xxxii, 33.
[3]Longcope, W. T.: Am. Jour. Med. Sc., 1902, cxxiv, 209.
[4]Cowern, E. W.: Jour. Am. Med. Assn., 1926, lxxxvi, 58.

OBSERVATION ON BLOOD CULTURES WITH A SPECIAL REFERENCE TO THE QUANTITY OF THE BLOOD USED*

By Herbert Fox, M.D., and William G. Leaman, M.D., Philadelphia, Pa.

THE following analyses have recently been made with the idea of discovering the usefulness of our blood culture technic, and since some of the observations may be helpful to others, it has seemed well to put our material on record. Comparison of data from one source of information with those from another may or may not be possible, since percentage results of blood culture success and failure depend upon many things. One of these factors, as we shall try to point out, is the quantity of blood used. Other factors of great importance are the types of cases tested, the activity or stage of the process, the technic and the media used, and the experience of the operator. Strict comparison can only be made when all factors are known and evaluated. Repeated cultures on the same case will increase the percentage of positives for a disease but may be misleading for the total of blood cultures taken. It is obvious that the oftener an attempt is made the greater is the probability of discovering circulating bacteria. So too, we think, it will be admitted that the greater the amount of blood taken, the greater the chance of growing bacteria therefrom; our data show an increase of findings when this has been done. Blood cultures taken by the usual technic are analyzed for the purpose of recording routine results. When, however, the examination has been negative in the presence of evidence suggesting a bacteremia, a large quantity of blood has been withdrawn, the amount safe for the patient being decided in conference between the clinician and the laboratory man. Repeated cultures may be necessary even by this method, but a negative result certainly has a greater significance.

A series of 321 cultures was made by the following simple method: From 10 to 15 c.c. of blood were withdrawn and blood agar plates and broth bottles inoculated in the usual way at the bedside. Of the 321 cultures made in this way 65 or 25.4 per cent were positive and 256 or 74.6 per cent negative.

Typhoid.—There were 27 cultures made from the blood of patients diagnosed typhoid fever clinically and who showed a positive Widal reaction at one stage or other of the disease. Of the 27 cultures 12 or 44.5 per cent were positive and 15 or 55.5 per cent were negative. They were all grown in bile enrichment media.

Pneumonia.—There were 58 cultures of the series made on pneumonia cases of various types on the medical side of the hospital and from 16, or 27 per cent of these cases the pneumococcus was recovered. Five were Type I; four were Type II; one Type III and six Type IV.

Puerperal Sepsis.—There were 56 cultures of the series made in the Maternity Wing on cases diagnosed sepsis. Of these 17 or 30.3 per cent were positive and 39 or 69.6 per cent negative. Streptococcus pyogenes appeared 7 times; Staphylococcus aureus (hem.) 6 times,

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Staphylococcus albus (hem.) 1, Streptococcus equinus 1, Streptococcus mitis 1, and Streptococcus subacidus 1. The streptococci were classified according to Holman's classification, which we have found very satisfactory.

Endocarditis.—There were 15 cultures taken in the Medical Wards during the past two years on cases having all the clinical characters of endocarditis. Five or 33.3 per cent were positive. A streptococcus of the viridans type was obtained 3 times, a Streptococcus mitis 1, and a Streptococcus pyogenes 1.

Meningitis.—There were 11 cultures made on meningitis cases and 4 positives resulted from this series or 27.4 per cent. The meningococcus was obtained twice, a streptococcus once and yeast was grown from another case.

Simple Blood Culture.—In 28 cases we failed to reveal any organism in the blood stream where the procedure was requested to try to determine the case of pyrexias of unknown origin.

Reference to Table I will show that no positive results were obtained by culturing the blood of 20 cases clinically diagnosed chronic arthritis.

For some time past we have directed our attention to the method already mentioned which depends upon taking a large quantity of blood and which

Fig. 1.—Bottle as prepared for use can be assembled cheaply in any laboratory.

we speak of as the *massive method* to emphasize the amount to be withdrawn from the vein. Fifty cubic centimeters have been the minimum, but many times 100 c.c. or more have been taken. This blood is collected into a 200 c.c. wide mouth bottle equipped with a tightly fitting rubber stopper with 2 perforations. Through each hole passes a glass connection, one going to the needle, the other to a rubber tubing fitted with a glass mouthpiece through which suction is made by the operator to facilitate the flow of blood from the vein. The blood is collected in the usual way in the bottle containing 100 c.c. of sterile doubly distilled water and 3 gm. of sodium citrate. On referring to the literature we find that Lintz in 1913 described a similar but somewhat less simple apparatus.

After the blood is drawn into the bottle, which is concealed from the patient's view under a sterile cover, it is taken directly to the laboratory for inoculation into media. This avoids a display of test tubes, media, etc.,

at the patient's bedside. Indeed, so simple is the collection and transportation of blood by this method that it lends itself to the use of the general practitioner with whom blood cultures have, on the whole, failed to become popular. In the laboratory the blood is placed in large tubes and centrifuged for twenty minutes at high speed. Under the greatest precautions the sediment is used for seeding into media. The blood will be defibrinated and almost completely laked by the citrate water. The broken cells will act as a mechanical filter and drag to the bottom the bacteria in the blood. Transfer of sediment to culture media may be done in one of two ways: the supernatant liquid may be poured away leaving at the bottom a fairly solid layer of reddish-gray material, or a sterile pipette may be plunged through the upper layers and the sediment collected in it. A long tubing with a glass

Fig. 2.—Bottle wrapped as sterilized for use.

mouthpiece, attached to the pipette, facilitates the removal of the sediment. Deep serum water broth, plain and blood agar plates constitute the ordinary media used. In cases where we suspected the typhoid-colon group, bile enrichment media have been used. Glucose broth has also been used to advantage. Brain medium has been used at times but with no conspicuous advantage.

We have had an opportunity to take 34 blood cultures by this so-called *massive method*. We report 14 positive results or 41 per cent and 20 negative or 59 per cent. The cases upon which this method was used, those with previous negative results or with little promise of positive outcome by the ordinary technic, consisted of subacute endocarditis, pelvic inflammation, pyelitis, tonsillitis, acute respiratory infections, chronic arthritis, Hodgkin's disease and neoplasms.

TABLE I

SUMMARY OF 321 BLOOD CULTURES BY THE SIMPLE METHOD

DIAGNOSIS	POSITIVE	NEGATIVE	PER CENT POSITIVE
Typhoid	12	15	44.5
Meningitis	4	7	36.3
Pyonephroses	1	1	50
Puerperal Sepsis	17	39	30.3
Endocarditis	5	10	33.3
Pneumonia	16	42	27.0
Septicemia	4	12	25.0
Phlebitis	1	2	33.0
Rheumatic Fever	1	4	20.0
Mastoiditis	3	7	30.0
Cellulitis	1	7	12.5
Parotitis	0	2	00.0
Erysipelas	0	3	00.0
Pyrexias Unknown Origin	0	28	00.0
Purpura	0	12	00.0
Pernicious Anemia	0	10	00.0
Chronic Arthritis	0	20	00.0
Empyema	0	2	00.0
Post Operative Wound Infection	0	18	00.0
Pyelitis	0	15	00.0
	65	256	

TABLE II

MASSIVE BLOOD CULTURES

DIAGNOSIS	POSITIVE	NEGATIVE	PER CENT POSITIVE
Subacute Endocarditis	4	1	80
Chronic Arthritis	2	4	33.3
Hodgkin's Disease	1	2	33.3
Lymphosarcoma	1	1	50
Pyelitis	3	1	75
Respiratory Infection	1	2	33
Rheumatic Fever	0	2	0
Mononucleosis	0	2	0
Pelvic Inflammation	1	1	50
Tonsillitis	0	2	0
Chronic Osteomyelitis	1	2	33
	14	20	

This technic gives, therefore, about 16 per cent better results than does the simple routine method. Its value is especially definite in cases of endocarditis where we obtained 80 per cent of positive cultures. This figure can almost certainly be improved upon.

A diphtheroid was obtained from a patient suffering from Hodgkin's disease and from a case of lymphosarcoma. Cases of acute pyelitis yielded positive cultures in three out of four instances or a percentage of 75. Colon bacillus was found once; B. mucosus capsulatus once and a hemolytic Staphylococcus once. The discovery of a number of the B. capsulatus group is most unusual and worthy of record. Rhea and Emmons also report its finding. It is not, however, characteristic for this organism to invade the blood stream, although it doubtless plays an important rôle in carrying on inflammation of the pelvis and of the bladder whence it might occasionally enter the circulation. The colon bacillus was also obtained once from a case of pelvic suppuration; a subsequent culture three weeks later was done on this case and was negative.

The patient was clinically improved, and we had no reason to doubt our original finding.

Perhaps the most interesting of our findings occurred in an acute respiratory tract infection, resembling influenza complicated by tonsillitis. From this blood by the *massive technic* there grew Streptococcus mitis, Micrococcus catarrhalis, and a diphtheroid. A repetition of the culture four days later resulted in no bacterial growth whatever. The streptococcus was identified by the Holman scale, the M. catarrhalis by the Elser-Huntoon criteria. The finding of this coccus in the blood is most unusual and though undoubted, is held *sub judice* until more data are available. Canon (Bakteriologie des Blutes, 1905) credits the possibility of the entrance of this gram-negative group from the pharynx into the blood.

A case of chronic osteomyelitis with discharging sinuses yielded a hemolytic Staphylococcus aureus, whereas two similar cases were negative. Results were negative in two cases of acute tonsillitis with adenitis.

We do not wish to draw conclusions from this short series of blood cultures but we venture to suggest that greater satisfaction will be experienced by both clinicians and laboratory workers if larger quantities of blood are used, especially if the bacterial contents be concentrated by hemolysis, defibrination and centrifuging. The method and the bottle that we describe are less cumbersome than others given in the literature and are practicable for clinicians in private practice if they will take a very little extra trouble. Certainly it seems from our experience that a higher percentage of findings will result and that a greater variety of bacteria will be discovered by the use of a large quantity of blood. Such a technic may obviate a repetition of blood culture. The growth of bacteria is no more rapid by this method; but, of course, when a larger number are present in the original seeding, the mass of growth is greater and more quickly perceptible. By this technic negative cultures seem to have more significance.

REFERENCES

Edsall: Clinical Value of Blood Cultures, Pennsylvania Med. Jour., 1905-1906, ix, 844.

Fowler: A Short Note on Blood Cultures, Jour. Roy. Army Med. Corps, Lond., 1912, xviii, 574.

Gerstley, Jesse R., and Fuller, Harry B.: A Simple Technic for Blood Cultures, Jour. Am. Med. Assn., 1914, lxii, 376.

Hefferman and Clarke: Blood Culture and Clinical Diagnosis, Jour. Royal Army Med. Corps, Lond., 1915, xxv, 229.

Hubst: The Value of Blood Culture in the Diagnosis of Infectious Disease, Long Island Med. Jour., 1913, vii, 7.

Judd and Simon: The Vacuum Tube of Keidel as Applied to Blood Culture Work, Jour. Am. Med. Assn., 1915, lxiv, 822.

Libman: General Infections by Bacteria, Jour. Michigan Med. Soc., November, 1924, xxiii, 462.

Lintz: Blood Cultures Simplified by a New Apparatus, Long Island Med. Jour., 1913, vii, 60.

Macleod, Bevan, and Brown: The Technic of Blood Culture, Jour. Path. and Bacteriol., 1918, xxii, 74.

Mann, Rainsford, and Warren: Report of Blood Cultures Examined in the Pathological Laboratory of the Roosevelt Hospital, Med. and Surg. Rep. Roosevelt Hosp., 1915, 231.

McJunkin: A Tube for Collecting Blood for Cultures, Jour. Am. Med. Assn., 1914, lxii, 774.

Rhea, Lawrence J., and Emmons, Arthur B.: A Series of 91 Consecutive Blood Cultures, Boston Med. and Surg. Jour., 1907, clvii, 809.

Rythenberg, Charles: Value of the Use of Ammonium Oxalate in Blood Culture-Technic, Proc. N. Y. Path. Soc., 1908-1909, N. S., viii, 192.

Rythenberg, Charles: Jour. Med. Research, 1909, p. 79.
Straus, Aubrey H.: Blood Cultures as a Diagnostic Aid, Virginia Med. Month., January, 1924, l, 701.
Straus: Significance of Negative Blood Culture Depends on Method Used, JOUR. LAB. AND CLIN. MED., February, 1924, ix, 345.
Straus and Bird, L. C.: Practical Use of Blood Culture in Public Health Work, Am. Jour. Public Health, May, 1924, xiv, 413.
Thornley: Notes on Blood Culture Technic, Brit. Med. Jour., 1916, xi, 555.
Weston, Paul G.: A Simple Method for Blood Culture, Jour. Am. Med. Assn., 1916, lxvi, 507.

NOTE ON THE QUANTITATIVE DETERMINATION OF ARSENIC IN ORGANIC MATERIAL*

BY ALLAN WINTER ROWE, PH.D., BOSTON, MASS.

A PROCEDURE frequently requested in the conduct of the general hospital laboratory is the determination of arsenic in body fluids or in other organic material. The amounts present are seldom large; and in certain parts of the country, at least, traces of arsenic are to be regarded as a normal constituent of the substances in question. It is possible in a district such as New England that the widespread use of arsenate of lead in combating the ravages of the gypsy moth is responsible for the condition. Equally it may be that minute quantities of arsenic are intrinsically a normal constituent of the human body as claimed by Gautier. In any case, with arsenic possibly present as a normal constituent, only quantitative methods are of value and these to ascertain if the amount of the offending material observed be outside what may be designated as normal limits. The present method here presented is an adaptation of the method first defined by Sanger and Black[1] and later modified by W. A. Boughton.[2] It is presented here in this form primarily for the benefit of those who desire a sensitive, accurate, and ready means of quantitating arsenic in the presence of much organic material.

This method consists primarily of two independent procedures, one being the extraction of the arsenical material and the other the actual estimation of the arsenic.

EXTRACTION

The entire amount of urine or body fluid to be examined is concentrated on a steam bath, to a volume of 50 to 75 c.c., and a sufficient amount of arsenic-free hydrochloric acid is added to form the constant boiling mixture of the acid solution (20 per cent HCl solution).

The practice in this laboratory is to carry out the evaporation in a porcelain or Pyrex evaporating dish and to wash the concentrated material into the distilling flask of the apparatus shown in Fig. 1, first with a small amount of water and then with the hydrochloric acid.

The solution is now boiled, and the distillate is collected under an amount of ammonium hydroxide slightly larger than the theoretical amount required

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to neutralize the hydrochloric acid distilled over (about 100 c.c. of 28 per cent NH_4OH). At the conclusion of the operation the stopcock is opened to release the negative pressure and prevent sucking back of the distillate.

This method with a slight modification can be used for the extraction of arsenic from such solid organic material as finely chopped muscle, hair, felt, and similar substances. The process is modified merely in that the arsenic-free hydrochloric acid of the highest concentration (about 37 per cent) is added directly to the organic material in the distillation flask without dilution in order to liberate the arsenic content. In using the strong

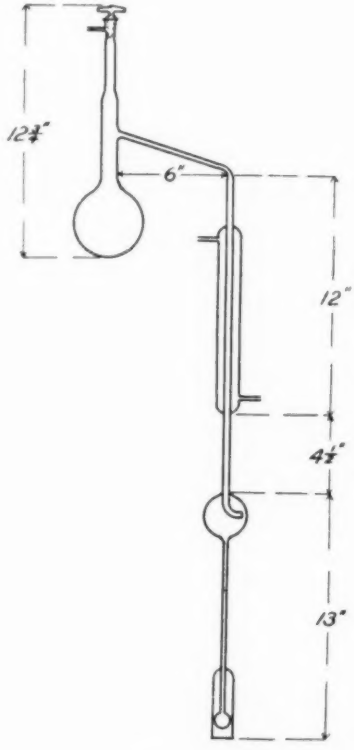

Fig. 1.

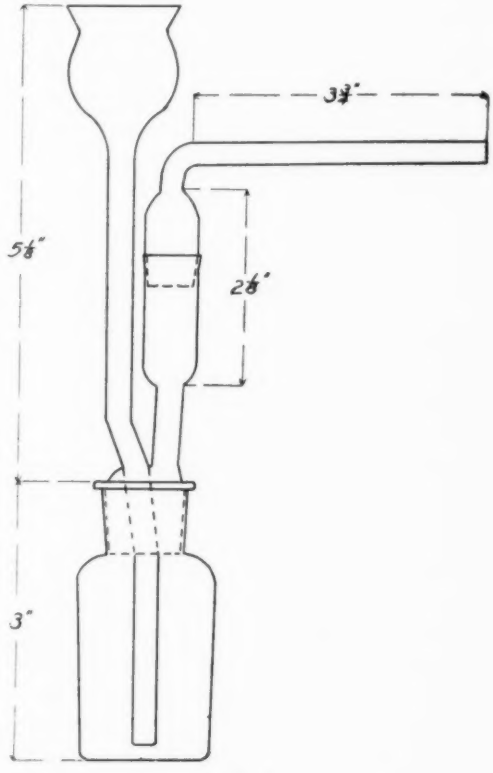

Fig. 2.

acid it may be necessary to attach an ammonium chloride absorption tube to the ammonium hydroxide receiving apparatus in order to avoid the possibility of mechanical loss of arsenic trichloride. The absorption tube is not needed when distilling a constant boiling acid mixture as very little gaseous hydrochloride is evolved.

The arsenic is distilled over as the chloride and presumably forms ammonium arsenite as expressed by the equation

$$AsCl_3 + 6NH_4OH = (NH_4)_3AsO_3 + 3NH_4Cl + 3H_2O$$

The arsenic is now precipitated from solution by the addition of 2 to 3 c.c. of saturated $Fe_2(SO_4)_3$ and filtered. The precipitate is dissolved in the

cold in as small amount of 10 per cent HCl as possible and transferred to the hydrogen generator.

The operations thus involved may be represented diagramatically by the equations

$$(a)\quad 2(NH_4)_3AsO_3 + Fe_2(SO_4)_3 = 2FeAsO_3 + 3(NH_4)_2SO_4$$
$$(b)\quad FeAsO_3 + 6\,HCl = FeCl_3 + AsCl_3 + 3H_2O$$

Another and equally tenable hypothesis assumes that ferric arsenite is too soluble to allow the precipitation and collection of the very small amounts of arsenic with which this method deals and that instead of the above reactions the arsenic may be adsorbed on ferric hydroxide formed when ferric sulphate is added to the ammoniacal solution.

It has been found that magnesium hydroxide is almost as efficient as ferric hydroxide in the collection of arsenic. These observations are in perfect harmony with current therapeutic practice.

QUANTITATION

Three grams of uniformly granulated zinc are placed in the bottle of the hydrogen generator (Fig. 2) and a strip of sensitized paper (v.i.) in the 4.1 mm. deposition tube. A plug of absorbent cotton that has been kept over concentrated sulphuric acid and a loose plug of cotton that has been moistened with lead acetate and dried, are placed in the enlargements of the exit tubes. An hour's preliminary run is necessary to moisten the cotton partially. Add 15 c.c. of diluted hydrochloric acid (1-6), and let the hydrogen pass for ten to twenty minutes to make sure the reagents cause no stain. Add the whole or an aliquot part of the solution to be tested. Arsenic will produce a color on the paper in a few minutes, and this will reach a maximum within thirty minutes. The color of the arsenic bands may be developed either (1) by placing the paper in hydrochloric acid (1:1) for two minutes at a temperature of not over 60° C., or (2) by treating for a few minutes with concentrated ammonium hydroxide. The amount of arsenic is determined by comparison with standard bands prepared by treating test paper by operating the apparatus, having added definite amounts of arsenious oxide, and developing the color as above.[1]

The sensitized paper is prepared by soaking strips 4 mm. wide, of a hot pressed-paper made by Whatman, in a 5 per cent solution of recrystallized mercuric chloride. These are dried, cut into strips 7 cm. long, and protected from light and moisture in a stoppered bottle containing granular calcium chloride, covered with cotton.

It is needless to say that throughout these several operations not only must every care be exercised to avoid contamination of material but that all of the several reagents must be carefully tested to exclude the presence of arsenic in each of them. The method as here indicated is rapid, highly sensitive, and apparently accurate to a high degree, as shown by the recovery of known amounts of arsenic.

REFERENCES

[1]Sanger and Black: Proc. Am. Acad. Arts and Sc., 1907, xliii, 297.
[2]Boughton: Personal communication, The method described here is essentially that developed by Mr. Boughton. We take great pleasure in acknowledging our basic indebtedness to his generous suggestion and cooperation.

THE INFLUENCE OF MAGNESIUM SALTS ON AMBOCEPTOR AND COMPLEMENT TITRATIONS*

By Wilfred H. Kellogg, M.D., and L. Amy Wells, A.B., Berkeley, Calif.

EVERY serologist can recall occasions of failure in satisfactory clearing of tubes that should show complete hemolysis in the Wassermann test and also of trouble with unsatisfactory titrations of complement and amboceptor. The unexpected and often unexplainable fall in the usual titer of hemolysin has been particularly troublesome in systems employing a light cell suspension as in the Kolmer method.

We have uniformly traced our difficulties to salt solution and have solved them temporarily by trying different brands of salt and sometimes different lots of the same brand; we have often noted that a chemically pure product may not give as good results as a commercial product. Mason and Sanford[1] consider that the P_H of the saline is the important factor. This seemed very plausible, and for a time we regarded this explanation as adequate, but as time went on, we were unable to correct our difficulties from this angle. That the P_H alone is not much of a factor is suggested by some of the experimental titrations in the article already referred to. In their Table I a salt solution made from tap water not boiled, with a P_H of 7.8, gave an amboceptor titration of 1-6000, while a solution made with double distilled water (Table II) and having a P_H exactly the same gave a titration recorded as nil. The result here was evidently due to some difference between tap and distilled water, other than the mere matter of hydrogen-ion concentration. This difference we have found to depend on the presence of magnesium, which is in most natural waters. That a fairly wide range in the P_H of the salt solution is consistent with the occurrence of usual results is shown by the 1-3000 amboceptor titration obtained by the authors quoted (Table III) in an artificially acidified tap water solution having a P_H of 5.8. Incidentally, this experiment also shows the influence of magnesium in the tap water, for the titration of 3000 undoubtedly would not have been reached in such an acid medium without it.

We have performed many experiments with various lots of sodium chloride and have included variations in the P_H values as well as slight variations in the tonicity of the solutions and tap, distilled, and double distilled waters were used. There is nothing to be gained in tabulating these experiments, since nothing definite resulted beyond additional confirmation of the idea that reaction in itself is relatively unimportant. One particular lot of salt gave

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such uniformly high titrations that it was decided to search for a chemical difference between this and lots of sodium chloride. The outstanding characteristic of this brand of salt was found to be the presence of considerable amounts of magnesium. The next step was to analyze the tap water, which also gave high titrations with chemically pure sodium chloride, and it was found to contain 14 parts per million of magnesium. This quantity, estimated as chloride, represented 0.12 gm. per liter. Experiments with physiologic saline solution made with chemically pure salt, distilled water, and varying amounts of magnesium chloride showed that less than 0.02 gm. per liter were without effect or had little effect on the titration but that 0.05 and over markedly raised the titer of amboceptor over that obtained with salt solution not containing magnesium. The upper limit for the addition of magnesium chloride was reached when a mixture of isotonic sodium chloride solution and a mixture of isotonic magnesium chloride (19.0 gm. of moist crystals per liter) in the proportion of 98 parts of the former and 2 parts of the latter, was used. Any further addition of magnesium beyond this point resulted in a rapid fall in the titer of amboceptor until absolute inhibition of hemolysis

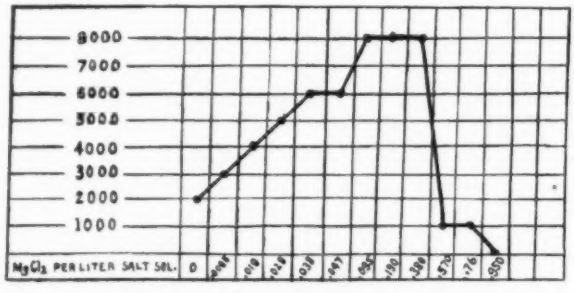

Fig. 1.—Amboceptor titrations.

was reached with 5 parts of the magnesium solution to 95 parts of normal salt solution. The influence of varying the content of magnesium chloride from minimum to maximum is graphically shown in the profile line chart (Fig. 1) in which the amount of magnesium present is shown in grams per liter.

The question naturally arose as to whether the favorable action of magnesium was due to the base itself, to the base in combination with certain acid radicals, or whether other bases might not also be effective. Accordingly various other salts were tried, their selection being influenced by such obvious associations or resemblances as chemical grouping with other metals, atomic weights, etc. Table I shows the results of titrations conducted with various magnesium salts, from which it appears that the base is the essential factor.

TABLE I

AMBOCEPTER TITRATION

	WATER	SALT	ADDED CHEMICAL*		AMBOCEPTER TITRATION
1	Distilled	Merck's C.P.	Magnesium Chloride	.08	1-8000
2	Distilled	Merck's C.P.	Magnesium Sulphate	.08	1-8000
3	Distilled	Merck's C.P.	Magnesium Citrate	.10	1-8000
Control	Distilled	Merck's C.P.	none		1-3000

*Per 1000 c.c.

Manwaring[2] noted the inhibiting action of certain salts, including $MgCl_2$, and found that no change of the corpuscles or amboceptor occurred and that by precipitating out the inhibiting salts, the hemolytic power of the mixture was restored; this showed that the complement was not destroyed. He considered that the action was due to the formation of simple ion-complement compounds that are hemolytically inactive. Similar conclusions are reached by Hektoen and Ruediger.[3] These observations may have a bearing on the question in strengthening our opinion that the action of magnesium in favoring hemolysis is not due to any chemical or physical action on the red cells.

TABLE II

	WATER	SALT	ADDED CHEMICAL GM. PER LITER		AMBOCEPTER TITRATION
1	Distilled	Merck's C.P.	Calcium Chloride	.08	1-3000
2	"	"	Calcium Chloride	.20	1-3000
3	"	"	Ammonium Chloride	.08	1-3000
4	"	Merck's U.S.P.	Zinc Chloride	.08	nil
5	"	"	Zinc sulphate	.08	nil
6	"	Merck's C.P.	Strontium Chloride	.08	1-1000
7	"	"	Copper Sulphate	.08	nil
8	"	"	Barium Nitrate	.08	1-2000
9	"	"	Manganese Chloride	.08	nil
10	"	"	Magnesium Chloride	.08	1-8000
11	Tap	"	None		1-8000

Table II shows the results of experiments with other salts. The explanation of the behavior of magnesium salts in the specific hemolysis of red blood cells is a subject for speculation and further investigation. We have at present no explanation to offer. The idea of catalysis suggests itself, but in this type of reaction it would be an unfamiliar manifestation of such an influence. Some chemical weakening of the erythrocytes with increased vulnerability to lytic agencies is also to be thought of. Magnesium being one of the alkaline earth metals, a reaction with the fats or the fatty acids of the red cells might be the basis of the damaging (if it be so regarded) influence of the magnesium. No evidence, however, of such reaction has been observed; and the titrations with calcium, barium, and manganese help to dispose of this hypothesis. Tests have been made to determine whether or not there is a preliminary reaction between the magnesium and the red cells, permitting hemolysis to proceed after the removal of the magnesium to the full extent as would be the case if the latter had a chemical effect on the cells themselves. To test this point, sheep cells were washed and stored in magnesium saline (magnesium chloride or sulphate 0.08, sodium chloride 8.42, distilled water 1000). Such suspensions were centrifuged, the saline poured off, and the volume restored with plain distilled water saline. Titrations conducted with cells so treated showed no increased titer of hemolysin and therefore no evidence of a separate influence, damaging or otherwise, on the red cells. Cells were also washed and suspended in both plain distilled water saline and magnesium saline and stored in the ice box. No difference was noted in the permanence of the cells.

Since the proof of the pudding is in the eating, and in the present case we must continue for a while along empirical lines, we add a representative

series of Wassermann tests on syphilitic sera (Table III) conducted with both types of salines. The method used was the Kolmer modification, the test quantitative, and the results reported in the manner suggested by Kolmer.*

TABLE III

WASSERMANN TESTS

SERUM	PLAIN DISTILLED WATER SALINE AMBOCEPTOR TITRATION 1-3000	MAGNESIUM SALINE AMBOCEPTOR TITRATION 1-8000
111	43100	44100
114	32100	43100
195	41000	41000
238	10000	10000
212	21000	30000
239	42100	42100
189	31100	41000
221	44410	44400
207	44410	44400

From the evidence of the Wassermann tests alone it would appear that the chief reason for using magnesium saline was one of economy of hemolysin. This is not the only advantage. When using plain distilled water saline, the titrations were frequently erratic, varying from nothing to four or five thousand but usually about three thousand. More disconcerting was the occasional occurrence of incomplete clearing in the tests, notwithstanding the utmost care with complement, etc. Since using the magnesium saline, these troubles have disappeared, the titrations always going with the same lot of amboceptor to six or eight thousand and the tests clearing promptly and sharply.

Perhaps we are too optimistic, considering the short time the method has been in use in this laboratory, but we feel that results so far obtained justify the presentation of our observations to others.

CONCLUSION

Magnesium salts in proper amount added to physiologic saline solution aid in the specific lysis of red blood cells thus increasing the titer of amboceptor and of complement. The optimum amount for this purpose is 0.1 gm. of the chloride or sulphate to the liter of normal salt solution.

REFERENCES

[1]Mason and Sanford: JOUR. LAB. AND CLIN. MED., February, 1924, ix, 313.
[2]Manwaring: Jour. Infect. Dis., January, 1904, i, 112.
[3]Hektoen and Ruediger: Jour. Infect. Dis., May, 1904, i, 379.

*Five serum tubes are used in addition to the serum control. The quantity of serum in each test is graded from 0.1 c.c. to 0.0025 c.c. The figures used for reporting the results represent the degree of inhibition for each of the 5 tubes used in the test. Thus serum 111 with magnesium saline is 4plus in the first and second tubes, one plus in the third, and negative in the last two.

A NOTE ON THE CALCIUM CONTENT OF THE SERUM OF NORMAL ADULTS*

By Isadore Rosen, M.D., and Frances Krasnow, Ph.D., New York City

IN A preliminary study of the calcium content of sera from syphilitic patients, comparatively high results consistently obtained, made it seem necessary to check the values in sera from normal individuals. Serum samples were obtained from 50 medical students showing no clinical signs of abnormality, and the calcium was determined by the Kramer-Tisdall method.[1] The results were as follows:

Serial No.	Milligrams of Calcium in 100 c.c. Serum	Serial No.	Milligrams of Calcium in 100 c.c. Serum
1	12.9	26	12.1
2	11.6	27	11.3
3	11.7	28	11.5
4	11.0	29	11.3
5	12.3	30	11.2
6	12.1	31	11.4
7	12.9	32	11.4
8	12.7	33	12.1
9	11.9	34	11.1
10	12.2	35	10.8
11	11.9	36	11.2
12	11.5	37	12.1
13	13.2	38	11.9
14	11.1	39	11.6
15	11.1	40	10.9
16	11.5	41	11.8
17	11.3	42	11.6
18		43	12.0
19	11.3	44	11.5
20	11.6	45	11.6
21	11.8	46	10.7
22	12.3	47	11.0
23	12.0	48	11.3
24	11.8	49	11.4
25	11.9	50	11.6

The maximum figure noticed in this normal series was 13.2 mg. per 100 c.c., the minimum 10.7 mg. per 100 c.c., and the average 11.6 mg. per 100 c.c.

As a check on the accuracy of our technic, parallel determinations were made on several of our sera through the courtesy of Miss Helen Rivkin of the New York Nursery and Child's Hospital and of Dr. Benjamin Kramer. In two of our samples showing by our analyses 11.90 and 11.47 mg., Miss Rivkin obtained 12.18 and 11.27 respectively. In 4 samples, giving 9.80, 11.50, 10.20, and 10.20 mg. in our series, Dr. Kramer obtained 10.20, 10.77, 9.92, and 10.68 mg. respectively.

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CONCLUSIONS

1. The calcium content of normal serum (10.7 to 13.2 mg. per 100 c.c.) was found to be definitely higher than the value usually accepted (9 to 11 mg. per 100 c.c.).

2. Of 50 normal individuals 7 per cent had a serum calcium content between 10.0 and 10.9 mg. per 100 c.c., 67 per cent between 11.0 and 11.9 mg. per 100 c.c., 24 per cent between 12.0 and 12.9 mg. per 100 c.c. and 2 per cent above 13.0 mg. per 100 c.c.

3. Ninety-one per cent of these cases had between 11.0 and 12.9 mg. of calcium per 100 c.c. of serum, while only 7 per cent had less than 11.0 mg. and none less than 10.0 mg.

REFERENCE

[1]Kramer, B., and Tisdall, F. F.: A Simple Technic for the Determination of Calcium and Magnesium in Small Amounts of Serum, Jour. Biol. Chem., 1921, xlvii, 475.

THE EFFECT OF PARAFFIN AND OILY SUBSTANCES UPON FILTER CANDLES*

By W. L. Holman, M.D., Toronto, Canada, and F. M. Krock, M.D., Baltimore, Md.

A STUDY of bacterial filtration, started some years ago at Stanford University and later at Johns Hopkins University, is being continued by one of us (W. L. H.). The purpose of this paper is to call the attention of bacteriologic workers to a chance of error and to a possible new technic in filtration studies.

Our attention was first called to the effect of paraffin on filter candles while we were testing them by the method we have reported.[1] One of the candles showed the escape of air bubbles from one spot on the candle at a pressure very much below that found in a previous test. We were for some time at a loss to discover what had happened to produce this spot. Finally we concluded after trying a number of treatments that the candle had probably come in contact on the laboratory table with a little paraffin, used for sealing tubes, which had melted in sterilizing the candle and had penetrated the pores.

This filter candle, a new Berkefeld V-3, required an air pressure of 565 mm. of mercury before leakage occurred. After it had been accidentally "oiled" presumably by contact with paraffin on one spot (which showed white when immersed in water) and was being retested, air bubbles came through this white area at less than 200 mm. pressure. The filter was then placed in xylol for forty-eight hours and allowed to dry; the white spot was no longer visible when placed in water; and the test showed 500 mm. mercury pressure before air bubbles came through.

*From the Department of Pathology and Bacteriology, University of Toronto and the Department of Pathology and Bacteriology, Johns Hopkins Medical School.

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After this preliminary finding, several other filters were studied and various estimations were made as to the size of the pores, the space occupied in the dry candles by air, the amount of water absorbed, the capillary pressure, the rate at which water or a peptone solution passed through, and the time under various filtration pressures at which they became permeable to bacteria. Having obtained certain standards, we attempted to "oil" the filters by different methods. We tried petrolatum, paraffin oil, and mixtures of the two. It was difficult not to get too much of the material into the filter pores, and we found they tended to become plugged. In attempting to clean them with ether, chloroform, by boiling and other methods, the partly cleaned filters would sometimes allow the passage of bacteria which were held back by the filter before and after treatment. A mixture of equal parts petrolatum and paraffin oil was used in most of the experiments on oiling the filters, but in the further studies of this problem it is planned to apply petrolatum, paraffin, and similar substances in solution using a volatile solvent such as ether or xylol.

Without, at this time, giving the results of the estimations used in determining certain physical characteristics of the filters tested, the results obtained in filtration experiments will be given briefly. It should be mentioned, however, that our results confirm those obtained by Mudd[2] in that the rate of filtration is of more value in determining the efficiency of filters than the estimation of the diameters of the pore spaces. The formula given by Bechhold for this latter purpose gives a higher value for the intergranular diameters, as the air pressure showing bubbles decreases. In our experiments the bubbles first appeared in the areas where presumably the spaces were partly filled with petrolatum, but the Bechhold formula applied here would indicate larger spaces. Mudd found the same type of results for old filters contaminated with dyes and proteins. The difference between our experimental results and those of others is to be seen in the fact that the "oiling" of the filters, although it cuts down the flow of fluid, makes the filters more permeable for bacteria. The ordinary plugged filter is as a rule less permeable for fluid and solids (including bacteria).*

A motile culture of B. prodigiosus, showing no viscid characters on media, was grown for twenty-four hours at room temperature in large flasks of Dunham's peptone solution. Only twenty-four hour cultures were used throughout, and the medium showed only a very faint cloud at this period. The apparatus employed enabled the removel of the filtrate at stated intervals, and all of the filtrate was used to test for the presence of bacteria; the collecting tubes, after being disconnected, were simply left at room tempera-

*In a personal communication from Stuart Mudd, he kindly called my attention to the fact that the Bechhold formula as used by him (Jour Bacteriol, 1923, viii, 459) contains an important error, and he has asked me to say that the estimated intergranular spaces given in his paper are ten times too small. The formula as given by Bechhold and used by Mudd was as follows: $D = \frac{4\beta \times 760}{p \times 1.033} \times 10^5$. Bigelow and Bartell showed (Jour. Am. Chem. Soc., 1909, xxxi, 1194) that the last term in the denominator should have been 10^4, not 10^5. Bechhold has acknowledged the mistake which will be corrected in the next edition of his book. This makes the intergranular diameters of the clean filters of all three types of the order of 4μ. This correction emphasizes the importance of the adsorption phenomena, since most of the common bacteria are well under 4μ in diameter. Probably the filters owe their usual tightness to three things: (1) to the tortuosity of the channels through them, (2) to prompt reduction of the intergranular spaces by adsorption of material from the filtering fluid, (3) to adsorption of the bacteria themselves in the filter. Perhaps there are other causes also.

ture for a week or more to allow growth to take place. Since only a slight opalescence was evident in the peptone solution at the time of filtration and since this microbe will after a few days develop a very opaque cloud and scum in this medium, it was felt that this was a safer procedure than if we transferred the filtrate to fresh tubes of medium. The bacteria in this early stage of growth are rapidly dividing and are probably much smaller in size than later when their dividing activity has become more sluggish. This method, we believe, puts the candles to a very rigid test.

Investigators frequently use cultures of B. prodigiosus without, as a rule, specifying the stage of growth, the medium used, or the physical condition of the bacteria. These are of great importance in filtration studies. We[3] have previously reported the successful filtration of a small anaerobic bacterium, resembling in morphology B. pneumosintes, through Mandler and Berkefeld N and W filters. This passage only occurred when the bacteria were grown in cooked meat medium without any addition of peptone or other nutrient material, and it failed to occur when the culture was taken from media containing peptone (Veillon agar, liver peptone broth). We consider that many of the bacteria are actually smaller under the conditions mentioned as they appear to be when examined microscopically.

The size of bacteria varies greatly not only from the effect of the available food but also with the stage of growth. Olitsky and Gates in a number of papers record successful filtration experiments with B. pneumosintes from nasopharyngeal washings in the first thirty-six hours of uncomplicated influenza and from a number of cultures presumably grown in ascitic-fluid rabbit-kidney medium with a petrolatum seal. Their paper[4] on the filterability of their organism gives no details of their method of filtration. They used Berkefeld V and N candles, but the culture used for testing these is not mentioned. In two of their animal passages, however, using filtered material, B. pyocyaneus was found in the lungs of the treated animals. This, they explained, was due to faulty technic in filtration in that the Berkefeld candle had been contaminated with this organism. We presumed from this that B. pyocyaneus was used as the test culture, and we, therefore, also used it in our experiments. In 1922[5] they reported striking morphologic changes of their bacterium when cultivated in dextrose peptone broth in that it became decidedly larger and more bacillary. The bacteria, however, reverted to the original minute form on cultivation in a dialysate of ascitic-fluid-rabbit-kidney medium. They did not give any filtration experiments on these cultures of larger forms, but it is presumed they would have been negative.

Mudd[2] in a very valuable contribution to the problems of filtration unfortunately used for controlling the filters a strain of B. prodigiosus which showed very pronounced sticky characters. He very kindly sent us his culture, and it corresponded to a very sticky strain which we had discarded as unsuitable for such a purpose. Hall and Howitt[6, 7] recently failed in numerous tests on the filterability of the minute anaerobe above mentioned. They used 2 of our strains and 24 of their own. Their procedure was different from ours in many details. The test culture B. prodigiosus was included in the material filtered; but since this organism is considerably larger than the little anaerobe

and if it were present in quantity, it would undoubtedly plug the filter pores. They used only 20 c.c. for each candle while we used highly diluted saline suspension in large amounts to prevent the plugging of the surface pores of the candles. Tests were made for bacteria using 1 c.c. of the filtrate which varied from 2 c.c. to 19 c.c., but we used the total filtrate collected at different intervals. The high pressure 637.5 mm. used by them would tend to stop the filters, especially if many bacteria are present in the fluid. We used pressures varying from 100 mm. to 400 mm. The time used (five minutes) is less than we used. We were particularly interested in comparing our organism with B. pneumosintes, and having no criteria from the reports of Olitsky and Gates as to control culture, dilution of material, pressure used, time of filtration or quantity of filtrate tested, we tried altering these various factors with the result that we obtained passage of our minute anaerobe through candles impermeable to cultures of B. pyocyaneus. This minute anaerobe is undoubtedly very close to the limits of filterability, and slight changes in size of the organism and conditions of filtration will affect the result. If size can be neglected, then most of the filtration experiments are absurd.

EXPERIMENTS WITH "OILED" FILTERS

We encountered some difficulty in the early attempts to remove the added petrolatum or oil. Mandler candle (2½ in.) No. 2, for example, which gave sterile filtrates after ten minutes at 100 mm. plus eight minutes at 200 mm. plus two minutes at 400 mm. of mercury with a total of 64.5 c.c. of medium, after having been cleaned, dried and immersed in paraffin oil at 53° C. for twenty-four hours, had the excess oil forced out by air pressure, was then autoclaved and tested with the above culture. The bacteria came through after a combined time of ten minutes at 200 mm. and twenty-five minutes at 400 mm. of mercury with 85 c.c. of the medium. The candle was then "cleaned" by passing ether through it for some time, thoroughly scrubbed with a brush, boiled, kept overnight in chloroform, again boiled for two hours and finally autoclaved. It was tested with a twenty-four hour culture, and the bacteria came through after ten minutes at 200 mm. pressure in 11 c.c. of medium, and at a pressure of 400 mm., 47 c.c. of medium which contained bacteria and showed an oily film on its surface was collected in four minutes.

To overcome this trouble it was thought possible to burn out the oil and petrolatum in a Wiesnegg furnace. The results were disastrous, for after this treatment a number of the candles were cracked, and the others showed loose collars. The preliminary tests on these candles showed, however, that the bacteria passed "oiled" and partially "cleaned" filters more readily than they did untreated candles. A number of examples will illustrate the results obtained.

Mandler candle (2½ in.) No. 4, which when new had withstood 100 mm. pressure for ten minutes plus 200 mm. for ten minutes plus 400 mm. Hg. pressure for seven minutes with a total filtrate of 67 c.c. was treated on one spot, about a square centimeter in size, with vaseline, was autoclaved and tested. Bacteria came through after five minutes at 200 mm. plus five minutes at 400 mm. mercury with 92 c.c. of medium. It was "cleaned" with alcohol and ether and gave bacteria under the same conditions in 80 c.c. of medium.

A further attempt at cleaning with ether, chloroform and prolonged boiling only resulted in reducing the amount filtered (and showing bacteria) to 75 c.c. in eight minutes.

Mandler candle (2½ in.) No. 5, gave 58 c.c. of filtrate free from bacteria after seven minutes at 100 mm. pressure plus seven minutes at 400 mm. mercury pressure. After treating with melted petrolatum, it was found almost plugged and required a 400 mm. Hg. pressure and a little heat to start it running. Bacteria were present in the 22 c.c. of the filtrate collected in fifteen minutes. After treatment with ether, chloroform, prolonged boiling and autoclaving, it gave 137 c.c. of sterile filtrate after twelve minutes at a pressure of 400 mm. Hg. We considered this candle to have been at this time free from the petrolatum (which it probably was), but it was heated in the furnace and broken.

Mandler candle (2½ in.) No. 7 was tested before treatment, and it gave 70.5 c.c. of bacterial free-filtrate after eight minutes at 100 mm. plus eight minutes at 400 mm. mercury pressure. One side of the candle was coated with petrolatum, autoclaved and tested. Bacteria came through in 15 c.c. of the medium after four minutes at 200 mm. pressure. It was "cleaned" in alcohol and ether and was retested. The bacteria grew in 35 c.c. of the filtrate obtained after five minutes at 200 mm. pressure. A further attempt at cleaning with ether, chloroform, and prolonged boiling resulted in some removal of the petrolatum. The test gave 84 c.c. sterile filtrate after four minutes at 200 mm. pressure plus three minutes at 400 mm., but the next 50 c.c. of filtrate after eight minutes at 400 mm. pressure contained bacteria.

Mandler candle (2½ in.) No. 8 gave before treatment 56.5 c.c. sterile filtrate after five minutes at 100 mm. plus five minutes at 400 mm. pressure. After twenty-four hours immersion in a mixture of equal parts petrolatum and paraffin oil, it was found to be almost plugged and required thirty minutes to obtain 75 c.c. of filtrate at a mercury pressure of 400 mm., but this contained bacteria. This candle after the ether, chloroform, and boiling treatment gave 142 c.c. of sterile filtrate after four minutes at 200 mm. plus six minutes at 400 mm. mercury pressure. This would indicate that it was practically clear of the oily substances, but the preliminary test had not determined what point of time or pressure or what amount of filtrate would allow bacteria to pass through it.

The results of the various tests on these 4 filters are tabulated for comparison in Table I. Three Berkefeld V candles were tested; but since they rapidly gave bacteria in the filtrates before treatment, they were not further studied. Two of the Mandler candles (No. 3 and No. 6) which had escaped the furnace were further studied in Tables II and III. It will be seen that these candles are apparently effective in holding back bacteria under the conditions used. Tables IV and V show that at a constant pressure bacteria pass through after ten to fifteen minutes with both candles. With candle No. 3 the amount of sterile filtrate reached 59 c.c. and with candle No. 6, 74.5 c.c. while the filtrate containing bacteria was between these amounts and for candle No. 3, 85 c.c. and for candle No. 6, 102.5 c.c. It will be seen that these amounts differ from those shown in Tables II and III where the time and the pressure were variable

Since it is believed that higher pressures may sometimes prevent bacteria from passing a filter which they go through under lower pressure, it was thought advisable to try higher pressures. The two filters were thoroughly cleaned before testing further. This was done by repeated washings with water, boiling for one-half hour in a 2 per cent washing soda solution, boiling in water for one hour with frequent changes of the water, washing by passing water through the filter and by scrubbing with a brush. The filters were then autoclaved and tested.

TABLE I

VARIOUS TESTS WITH 2½-INCH MANDLER FILTERS

FILTER NUMBER	TREATMENT	PRESSURE MM. HG.	TIME IN MINUTES	RATE PER MINUTE	AMOUNT IN C.C.	RESULT*
4	New	100	10	2.75	27.5	0
4	"	200	10	1.8	18.0	0
4	"	400	7	2.15	21.5	0
4	"	–	27	–	67.0	0
4	One small spot treated with petrolatum	200	5	8.0	40.0	0
4	" " " " " "	400	5	10.4	52.0	+
4	" " " " " "	–	10	–	92.0	+
4	Water, alcohol, ether. Spot still seen	200	5	7.0	35.0	0
4	" " " " " "	400	5	9.0	45.0	+
4	" " " " " "	–	10	–	80.0	+
4	" " " " " "	400	10	3.5	35.0	+
4	" " " " " "	–	20	–	115.0	+
4	Ether, boiled in water, $CHCl_3$ overnight, boiled in water two hours	200	5	5.0	25.0	0
4	"	400	3	16.6	50.0	+
4	"	–	8	–	75.0	+
5	New	100	7	3.28	23.0	0
5	"	400	7	5.0	35.0	0
5	"	–	14	–	58.0	0
5	Melted petrolatum. Almost plugged	400 Warmed to start flow	15	1.46	22.0	+
5	" " " "	400	15	2.66	40.0	+
5	" " " "	400	15	1.00	15.0	+
5	Ether, $CHCl_3$ and boiling	400	3	16.0	48.0	0
5	" " " "	400	3	13.0	39.0	0
5	" " " "	400	6	8.3	50.0	0
5	" " " "	–	12	–	137.0	0
7	New	100	8	3.7	29.5	0
7	"	400	8	5.1	41.0	0
7	"	–	16	–	70.5	0
7	One side with petrolatum	200	4	3.75	15.0	+
7	" " " "	400	11	tube broken		
7	" " " "	400	9	1.3	12.0	+
7	Water, alcohol, ether.	200	5	7.0	35.0	+
7	" " "	400	10	5.5	55.0	+
7	" " "	400	10	2.5	25.0	+
7	Ether, $CHCl_3$ and boiling	200	4	10.5	42.0	0
7	" " " "	400	3	14.0	42.0	0
7	" " " "	–	7	–	84.0	0
7	" " " "	400	8	6.25	50.0	+
7	" " " "	–	15	–	134.0	+
7	" " " "	400	9	3.9	35.0	+
8	New	100	5	4.1	20.5	0
8	"	400	5	7.2	36.0	0
8	Petrolatum and paraffin oil	400	20	2.1	42.0	0
8	" " " "	400	10	3.3	33.0	+
8	" " " "	–	30	–	75.0	+
8	" " " "	400	10	1.8	18.0	+
8	Ether, $CHCl_3$ and boiling	200	4	11.5	46.0	0
8	" " " "	400	3	16.6	50.0	0
8	" " " "	400	3	15.3	46.0	+
8	" " " "	–	10	–	142.0	+

*+ =Bacteria in filtrate. 0 = No bacteria in filtrate.

TABLE II

MANDLER CANDLE NO. 3

RESULTS OF FILTERING A TWENTY-FOUR HOUR CULTURE OF B. PRODIGIOSUS AT DIFFERENT PRESSURES

Time in minutes	10	10	12
Rate per minute in c.c.	2.2	2.05	2.166
Total in c.c. for each period	22	20.5	26.0
Total of fluid filtered in c.c.	22	42.5	68.5
Pressure in mm. mercury	100	200	400
Result	0	0	0

TABLE III

MANDLER CANDLE NO. 6

RESULTS OF FILTERING A TWENTY-FOUR HOUR CULTURE OF B. PRODIGIOSUS AT DIFFERENT PRESSURES

Time in minutes	7	7
Rate per minute in c.c.	3.4	4.3
Total in c.c.	24.0	30.0
Pressure in mm. mercury	100	400
Result	0	0

TABLE IV

MANDLER CANDLE NO. 3

RESULTS OF FILTERING B. PRODIGIOSUS AT A PRESSURE OF 200 MM. MERCURY

Time in minutes	5	5	5	5	5	5	5	5	10	5*
Rate per minute in c.c.	6.2	5.6	5.2	4.6	3.6	3.0	2.7	2.4	2.2	2.6
Total in c.c. for each period	31.0	28.0	26.0	23.0	18.0	15.0	13.5	12.0	22.0	13.0
Total of fluid filtered in c.c.	31.0	59.0	85.0	108.0	126.0	141.0	154.5	166.5	188.5	201.5
Result	0	0	+	+	+	+	+	+	+	+

*Pressure 400 mm. mercury.

TABLE V

MANDLER CANDLE NO. 6

RESULTS OF FILTERING B. PRODIGIOSUS AT A PRESSURE OF 200 MM. MERCURY

Time in minutes	5	5	5	5	5	5	5	5	10	5*
Rate per minute in c.c.	7.8	7.1	5.6	4.2	3.6	3.1	2.6	2.2	2.0	2.6
Total in c.c. for each period	39.0	35.5	28.0	21.0	18.0	15.5	13.0	11.0	20.0	13.0
Total of fluid filtered in c.c.	39.0	74.5	102.5	123.5	141.5	157.0	170.0	181.0	201.0	214.0
Result	0	0	+	+	+	+	+	+	+	+

*Pressure 400 mm. mercury.

Candle No. 3 was tested with a pressure of 650 mm. of mercury, and it will be seen no bacteria came through in 80 c.c. of medium in fifteen minutes. Candle No. 6 under a pressure of 200 mm. of mercury did not allow the passage of any bacteria in 98 c.c. of filtrate in fifteen minutes. It is suggested that the cleaner walls account for the difference between the results shown in Tables IV and V and those shown in Tables VI and VII. With these results as a basis, the two filters were then treated, after cleaning and drying, with a mixture of equal parts of petrolatum and paraffin oil, autoclaved and tested (using a constant pressure of 200 mm. of mercury). The results are shown in Tables VIII and IX.

The 2 candles permit the passage of bacteria in between ten and fifteen minutes filtration and an amount of between 22.5 c.c. and 30.5 c.c. for candle No. 3 and between 14.9 c.c. and 18.9 c.c. for candle No. 6. It is to be noted that the rate of filtration is much slower than in Table VII under the same pressure. The petrolatum-oil mixture tends to fill the passages, but

it also prevents the bacteria adhering to the space surfaces in the filter substance. There is a marked contrast between the results of Tables VI and VII and those of Tables VIII and IX. The 2 filters were now immersed in xylol for twenty-four hours then in antiformin for another twenty-four hours, distilled water was run through overnight (about fifteen hours) and they were then boiled in water. After attaching the candles to the filter cylinders, they were autoclaved, cooled, and tested.

TABLE VI

MANDLER CANDLE NO. 3, PRESSURE 650 MM. MERCURY

RESULTS OF FILTERING B. PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED BY WASHING, BOILING FOR A HALF HOUR IN WASHING-SODA, BOILING IN WATER FOR ONE HOUR WITH FREQUENT CHANGES, SCRUBBING AND AUTOCLAVING

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	3.0	9.0	10.0	9.0	8.0	7.0	5.0	5.0	5.0	4.0	4.0	2.75
Total in c.c.	3.0	12.0	22.0	31.0	39.0	46.0	51.0	56.0	61.0	65.0	69.0	80.0
Result	0	0	0	0	0	0	0	0	0	0	0	0

TABLE VII

MANDLER CANDLE NO. 6, PRESSURE 200 MM. MERCURY

RESULTS OF FILTERING B. PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED BY WASHING, BOILING FOR A HALF HOUR IN WASHING-SODA, BOILING IN WATER FOR ONE HOUR WITH FREQUENT CHANGES, SCRUBBING AND AUTOCLAVING

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	10.0	11.0	13.0	10.0	9.0	8.0	6.0	5.0	5.0	5.0	4.0	3.0
Total in c.c.	10.0	21.0	34.0	44.0	53.0	61.0	67.0	72.0	77.0	82.0	86.0	98.0
Result	0	0	0	0	0	0	0	0	0	0	0	0

TABLE VIII

MANDLER CANDLE NO. 3

RESULTS OF FILTERING B. PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED WITH A MIXTURE OF EQUAL PARTS PETROLATUM AND PARAFFIN OIL. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	5	5*
Rate per minute in c.c.	3.0	2.8	2.6	2.4	2.3	2.2	2.0	1.9	1.7	1.6	1.6	3.0
Total in c.c.	3.0	5.8	8.4	10.8	13.1	15.3	17.3	19.2	20.9	22.5	30.5	45.5
Result	0	0	0	0	0	0	0	0	0	0	+	+

*Pressure 400 mm. mercury.

TABLE IX

MANDLER CANDLE NO. 6

RESULTS OF FILTERING B. PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED WITH A MIXTURE OF EQUAL PARTS PETROLATUM AND PARAFFIN OIL. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	5	5*
Rate per minute in c.c.	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.2	1.0	0.8	0.8	1.8
Total in c.c.	2.0	3.9	5.7	7.4	9.0	10.5	11.9	13.1	14.1	14.9	18.9	27.9
Result	0	0	0	0	0	0	0	0	0	0	+	+

*Pressure 400 mm. mercury.

TABLE X

MANDLER CANDLE NO. 3

RESULTS OF FILTERING B. PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED IN XYLOL FOR TWENTY-FOUR HOURS; IN ANTIFORMIN FOR TWENTY-FOUR HOURS; WITH DISTILLED WATER PASSING THROUGH FOR ABOUT FIFTEEN HOURS; BOILED AND AUTOCLAVED. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	8.0	7.5	7.0	6.5	6.0	5.5	5.0	4.5	4.3	4.0	3.5	3.0
Total in c.c.	8.0	15.5	22.5	29.0	35.0	40.5	45.5	50.0	54.3	58.3	61.8	73.8
Result	0	0	0	0	0	0	0	0	0	0	0	0

TABLE XI

MANDLER CANDLE NO. 6

RESULTS OF FILTERING B. PRODIGIOSUS AFTER CANDLE HAD BEEN TREATED IN XYLOL FOR TWENTY-FOUR HOURS; IN ANTIFORMIN FOR TWENTY-FOUR HOURS; WITH DISTILLED WATER PASSING THROUGH FOR ABOUT FIFTEEN HOURS; BOILED AND AUTOCLAVED. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	10.0	9.3	8.7	8.0	7.5	6.9	6.0	5.5	4.5	4.0	3.5	3.25
Total in c.c.	10.0	19.3	28.0	36.0	43.5	50.4	56.4	61.9	66.4	70.4	73.9	86.9
Result	0	0	0	0	0	0	0	0	0	0	0	0

TABLE XII

MANDLER CANDLE NO. 3

RESULT OF FILTERING B. PRODIGIOSUS AFTER CLEANING AND "OILING" CANDLE AS IN TABLE VIII. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	2.0	3.0	3.0	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	1.8
Total in c.c.	2.0	5.0	8.0	11.0	14.0	17.0	20.0	22.5	25.0	27.5	30.0	37.2
Result	0	0	0	0	0	0	0	0	0	+	+	+

TABLE XIII

MANDLER CANDLE NO. 6

RESULT OF FILTERING B. PRODIGIOSUS AFTER CLEANING AND "OILING" CANDLE AS IN TABLE IX. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	4.0	4.0	4.0	3.5	3.5	3.5	3.0	3.0	2.5	2.5	2.0	1.25
Total in c.c.	4.0	8.0	12.0	15.5	19.0	22.5	25.5	28.5	31.0	33.5	35.5	40.5
Result	0	0	0	0	0	0	0	0	0	0	0	+

TABLE XIV

MANDLER CANDLE NO. 3

RESULT OF FILTERING B. PRODIGIOSUS AFTER CLEANING CANDLE WITH XYLOL FOR TWENTY-FOUR HOURS. NO ANTIFORMIN. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	4.0	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	6.75
Total in c.c.	4.0	10.0	17.0	24.0	31.0	38.0	45.0	52.0	59.0	66.0	73.0	100.0
Result	0	0	0	0	0	0	0	+	+	+	+	+

TABLE XV

MANDLER CANDLE NO. 6

RESULT OF FILTERING B. PRODIGIOSUS AFTER CLEANING CANDLE WITH XYLOL FOR TWENTY-FOUR HOURS. NO ANTIFORMIN. PRESSURE 200 MM. MERCURY

Time in minutes	1	1	1	1	1	1	1	1	1	1	1	4
Rate per minute in c.c.	6.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	7.0	7.0	7.0	6.5
Total in c.c.	6.0	14.0	22.0	30.0	38.0	46.0	54.0	62.0	69.0	76.0	83.0	109.0
Result	0	0	0	0	0	0	0	0	0	0	+	+

There are two indications in Tables X and XI that the filters have been freed of the mixture. First the medium comes through very much more quickly so that with No. 3, 73.8 c.c. is filtered in fifteen minutes in contrast to only 30.5 c.c. in the oiled candle (Table VIII) and with candle No. 6, 86.9 c.c. in contrast to 18.9 c.c. in the oiled filter (Table IX). Second no bacteria came through although 2.4 and nearly 4.6 as much medium was obtained in the same time as in the oiled filters. The results shown with clean filters in Tables VI and VII should also be compared. After thorough washing and

drying the two candles were treated as before with the petrolatum-oil mixture, autoclaved and tested.

In Tables XII and XIII it is seen that the filtration is slower and that bacteria pass through within the time limit of fifteen minutes. With candle No. 3 this occurred when 27.5 c.c. had filtered in ten minutes and with candle No. 6 after 40.5 c.c. had been collected in fifteen minutes. The results are in keeping with those shown in Tables VIII and IX, although it would appear that there was more plugging of the spaces in the results tabulated in Table VIII than in Table XIII and a trace less in candle No. 3 of Table XII. The filters were again treated with xylol for twenty-four hours but not with antiformin, autoclaved and tested.

Much more fluid came through both candles than in the previous experiments with "clean" filters, and bacteria passed the filter within the fifteen minutes of the test. It is to be noted, however, that with filter No. 3 (Table XIV) it required 52 c.c. before bacteria were found in contrast to the other tests on oiled filters, viz., 30.5 c.c. (Table VIII) and 27.5 c.c. (Table XII). It is true it only needed eight minutes to filter this 52 c.c. With filter No. 6, 83 c.c. containing bacteria came through in ten minutes; other experiments with this candle when "oiled" show a rate of 18.9 c.c. in fifteen minutes (Table IX) and 40.5 c.c. in fifteen minutes (Table XIII) with bacteria. It is believed, nevertheless, that our treatment with xylol alone did not completely remove the oily mixture from the filter.

This work is being continued, and a new series of candles is being carefully measured to determine the pore space so that it can be estimated how much of this is filled with the petrolatum, paraffin oil or paraffin when the dry candles are treated with these substances in solution in ether, xylol, benzene or other solvent. Measurements are also being collected on the capillary pressure, the amount of water absorbed, and the rates of flow for different fluids, so that the alterations resulting from the oiling may be more accurately determined.

The object of this research is to enable us to remove the adsorptive character of the surface walls of the intergranular spaces without interfering too greatly with the rate of filtration. It is well known that filtration through clay or porcelain candles does not result in merely a mechanical separation of bacteria due to their failure to pass the small interstices of the candle but depends on the action of complicated physical and chemical adsorption and other laws, and it is in an endeavor to lessen this adsorptive character that this study is being followed.

In filtering all manner of substances the loss of valuable constituents by their adhesion to the filter wall has been considered inevitable. The loss of antitoxin from antitoxic serums, the disappearance of enzymes which are supposed to be present in a fluid, the complete absence of virus after filtration experiments shown by testing with ground up filter to be in the filter substance, and the many other examples of similar phenomena make the development of a better method of filtration highly desirable. It is to be hoped when the technic for oiling the candles is made more perfect that some information may be obtained in filtration studies of smallpox and other viruses.

There is another equally important side to the problem and that is the danger involved in accidentally bringing petrolatum or other oily substance in contact with filter candles. If this occurs between the test for its efficiency and the actual filtration, the intermediate autoclaving causes a distribution of the oily substance over the intergranular surfaces of the candle and may lead to erroneous results. This is a danger to be avoided in the filtration of anaerobic cultures where petrolatum is employed on the surface of fluid media. Since petrolatum and paraffin are so widely used in laboratories for lubricating purposes, the chances of this accident occurring are multiplied.

CONCLUSIONS

1. There is a potential danger present in many laboratories of accidentally changing the character of filter candles by permitting them to come in contact with small amounts of petrolatum, paraffin and similar substances.

2. These oily substances render the candles more permeable to bacteria and somewhat less permeable to fluids. Air bubbles also pass such filters under lessened pressure.

3. Autoclaving and ordinary methods of cleaning do not remove such substances, and an active solvent, such as xylol, is necessary.

4. Oiled filter candles may be useful in the study of many materials where the loss of important constituents by adsorption to the filter walls is to be avoided.

5. The application of the Bechhold formula in determining the size of the pores of filters would make it appear that these are larger after treatment with oily substances when actually they must be smaller. It is useless, therefore, in determining the efficiency of such treated filters.

REFERENCES

[1]Krock, F. H., and Holman, W. L.: A Simple Apparatus for Testing and Washing Filter Candles, Jour. Am. Med. Assn., 1923, lxxx, 1142.

[2]Mudd, S.: The Penetration of Bacteria through Capillary Spaces. I. Motility and Size as Influencing Filterability through Berkefeld Candles, Jour. Bacteriol., viii, 1923, 459.

[3]Holman, W. L., and Krock, F. H.: Microorganisms Suggesting B. Pneumosintes from the Mouths of Man and Laboratory Animals, Am. Jour. Hyg., 1923, iii, 487.

[4]Olitsky, P. K., and Gates, F. L.: Experimental Studies of the Nasopharyngeal Secretions from Influenza Patients. II. Filterability and Resistance to Glycerol, Jour. Exper. Med., 1921, xxxiii, 361.

[5]Olitsky, P. K., and Gates, F. L.: Experimental Studies of the Nasopharyngeal Secretions from Influenza Patients. VIII. Further Observations on the Cultural and Morphological Characters of Bacterium Pneumosintes, Jour. Exper. Med., 1922, xxxv, 813.

[6]Hall, I. C., and Howitt, B.: The Anaerobic Bacteria of the Oral Cavity, Proc. Soc. Exper. Biol. and Med., 1925, xxii, 541.

[7]Hall, I. C., and Howitt, B.: Bacterial Factors in Pyorrhea Alveolaris: IV. Micrococcus Gazogenes, A Minute Gram-Negative Nonsporulating Anaerobe Prevalent in Human Saliva, Jour. Infect. Dis., 1925, xxxvii, 112.

THE STANDARDIZATION OF TUBERCULIN*

By Joseph D. Aronson, M.D., Philadelphia, Pa.

TO determine the value of the methods proposed for standardizing tuberculin, the strength of 10 samples of tuberculin was ascertained by means of the intradermic method, the complement fixation and the precipitin reaction. These results were compared with those obtained by the Bureau of Animal Industry employing the toxic dose of tuberculin as the criterion of potency.

The intradermic standardization was carried out on guinea pigs infected intraperitoneally, four weeks previously with a human strain of B. tuberculosis. The same dose of 4 different samples was injected intradermally at widely separate parts of the abdomen. The doses employed were 0.01, 0.001, 0.0001 c.c., diluted with normal saline to a volume of 0.1 c.c. Forty-eight hours later the results were read and recorded as A, B, C, or D, depending upon the degree of necrosis, edema, or redness.

To determine the strength of tuberculin by means of the precipitin reaction, amounts of tuberculin ranging from 0.02 c.c. to 0.0000005 c.c. were added to 0.2 c.c. of an immune serum prepared by repeatedly injecting a goat intravenously with a living avirulent strain of bovine tubercle bacillus. After four hours incubation, the results were read the next day and recorded as XXXX, XXX, XX, X, trace or negative.

The complement-fixation reaction was carried out by adding varying amounts of the different samples of tuberculin to a fixed amount of the same immune serum used in the precipitin reaction. After adding two units of fresh guinea pig complement and incubating the tubes for two hours, sheep cells and antisheep amboceptor were added; and the final results were read the next day.

A comparison of the results obtained by the different methods indicates that a close agreement exists between the results obtained with the intradermic method and that obtained by determining the toxic dose. On the other hand, the results obtained by means of the complement fixation or the precipitin reaction do not agree with those obtained with the first two methods.

This study indicates that the intradermic method can be utilized to standardize tuberculin, and we suggest that a tuberculin to be considered of standard strength should produce a definite edema and redness with 0.001 c.c. of the tuberculin.

*From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.
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LABORATORY METHODS

A NEW TYPE OF MOTOR DRIVEN LONG PAPER KYMOGRAPH*

By D. E. Jackson, Ph.D., M.D., Cincinnati, Ohio

THIS kymograph has been designed to meet all ordinary requirements in the way of a long paper kymograph not only for student use but also for the most exacting research work. A very large range of adjustments and a number of special, newly designed facilities are embodied in the construction of the kymograph.

The instrument is driven by a small motor which runs on either direct or alternating current of 110 volts, thus providing power continually so long as current is supplied. The kymograph is compact, sufficiently heavy to resist vibrations and is made of metal throughout. It is only about two-thirds as large as the ordinary makes of long paper kymographs.

The main supporting stand (1) is made of heavy angle iron firmly held together by crossbars at the bottom and by two heavy iron plates (2 and 3) at the top. A crank (4) turns a worm which engages in the gear (5) which is mounted on a cylinder on which is wound the small steel cable (6) by means of which the main I-beam and the drums may be easily and quickly raised or lowered. The lower ends of the cable (one on either side) are attached to the lower end of the square main supporting shaft (23) on which is pivoted (at 7) the square iron block (24) to which is hinged (at 9) the main I-beam and also the semicircular, slotted plate by means of which lateral adjustment of the drums on a horizontal axis may be made in any position from horizontal to perpendicular on either side by means of the bolt (8) which is turned by a small rod passing through the bolt head. At (10) a rod passes through the head of a bolt which locks the main I-beam in any desired position as turned on a perpendicular axis.

The motor (11) by means of the belt (22) actuates a double series of worm and spur gears which greatly reduce the speed of the motor and give a series of ten changes in speed. The shifts from one to another of these changes can be quickly and easily made (while the motor is running if desired) by means of the two levers (14 and 15) and the lock knob (13). The belt (16) rides on two small cone pulleys which serve to double the ten rates of speed produced by the gears alone, thus making twenty rates of speed for the shaft on which is mounted the worm gear which turns the wheel (17) which, by means of a set screw, is adjustably mounted circularly on the shaft of the right-hand drum. By loosening the set-screw the drum is thrown out of gear and can be easily

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and independently turned by means of the crank (19). In this way the paper, which is pasted around the two drums while they are turned down in a horizontal position, is smoked, thus avoiding the use of a special drum smoker. Tightening the above set-screw again throws the drum in gear and transmits (through the wheel 17) the full twenty different speeds to the drum. If it is especially desired two extra cone pulleys can be attached to the motor and to the main gear wheel drive shaft, thus doubling the twenty previous speeds and

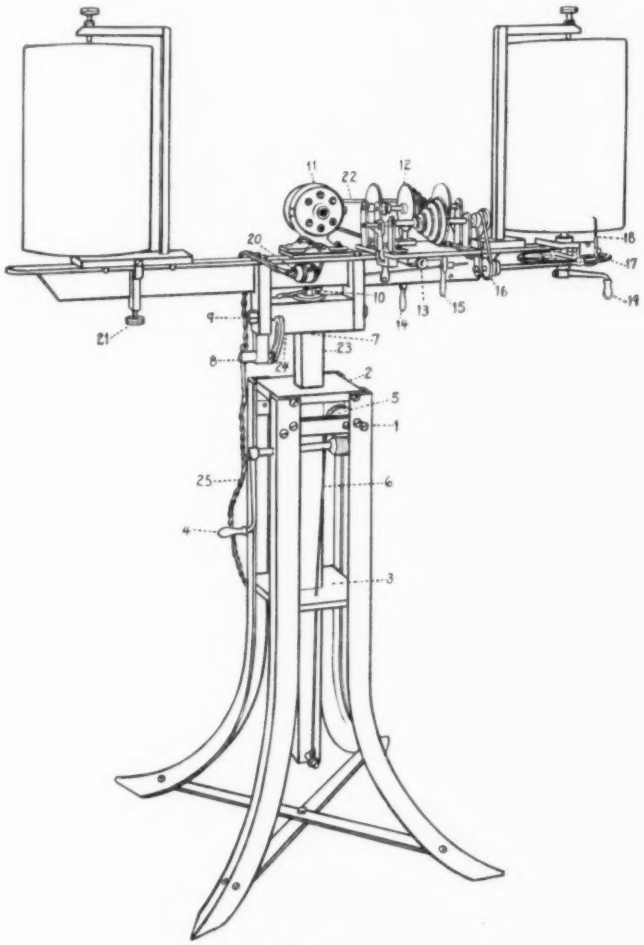

Fig. 1.

giving forty speeds to the drum. It is also possible to attach a special mechanical spinning device to the right-hand drum if desired.

The switch (20) controls the current which reaches the motor through the extension cord (25) which attaches to any lamp socket.

At (18) is shown a special time marking device which is run directly by the main drum driving mechanism. This time marker is exceedingly convenient and can be readily raised or lowered, or turned off the drum altogether at any moment desired. It is only as accurate as the speed of the motor which may

vary one per cent or more but in almost all instances is less liable to change and error than are other elements involved in the usual type of experiments performed. At any time the marker (18) can be turned off the drum and any other desired time recorder can be substituted. This change, however, I have never found it worth while to make in my own work.

The drums are full twelve inches high and eight inches in diameter. A record eight or nine feet long and one foot wide can thus be made. The position of the left-hand drum can be adjusted by set-screw (21).

The lock bolt (10) can be readily turned out entirely thus permitting the main I-beam and the drums to be lifted off of the main supporting stand.

Just below knob (13) and extending behind lever (15) is an extra long writing point which can be substituted for writing point (18), thus marking the time record up to the middle of the drum or higher in case two or more rounds of tracings are taken on the same long strip of paper (as would usually be the case).

This kymograph has been mainly constructed for me by Mr. George Grathwohl, mechanician in the department of pharmacology. The kymograph may be purchased from the Max Wocher & Son Company of Cincinnati, Ohio.

AN ADJUSTABLE SPHYGMOSCOPE FOR THE RECORDING SPHYGMOMANOMETER*

By Joseph Erlanger, M.D., St. Louis, Mo., and W. J. Meek, Ph.D., Madison, Wis.

EVER since the difficulty first arose of obtaining suitable rubber bulbs for the sphygmomanometer devised by one of us,[1] and it became obvious that some other type of sphygmoscope would have to be developed if the instrument was to be kept available for further use, tests have been in progress in an effort to find a suitable substitute. While engaged in this search, one of us (Meek) found that a clamped-off segment of Gooch crucible tubing was satisfactory for the purpose. After trying out a variety of samples a form of rubber known in the trade as "band tubing" was finally found which not only is satisfactory but in addition permits of certain adaptations which make it superior in three respects to the rubber bulb originally employed. These advantages consist: first, in the possibility of utilizing in the construction of the sphygmoscopic parts of the instrument, materials most of which are quite readily obtainable, if, indeed, they are not to be found on hand in many experimental laboratories; second, in being able, by means of a simple expedient, to adapt, within fairly wide limits, the sensitivity of the instrument to the pressure range of the subject under observation; and third, on account of this greater sensitivity, in being able to substitute for the compound recording lever with its relatively high inertia, a

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lever of simple construction and low inertia. This paper describes the means whereby these ends are accomplished and also certain modifications in the construction of the sphygmomanometer that have been made in this laboratory in recent years with a view to simplification of the adjustments necessary to adapt the instrument to changing and special conditions.

THE SPHYGMOSCOPE, SIMPLE FORM

Fig. 1 is a diagram showing the new sphygmoscope in a form that can be put together in any laboratory. *A* is a piece of the band tubing (No. 12, 1½ inch, black rubber) 14 to 15 cm. in length, which is drawn over a brass tube, *B*, of such an outside diameter (0.97 inches) that the rubber *just is not stretched* by it. If this fit be too tight, so that more than 30 mm. Hg of distending pressure becomes necessary to lift the rubber from the cylinder, it

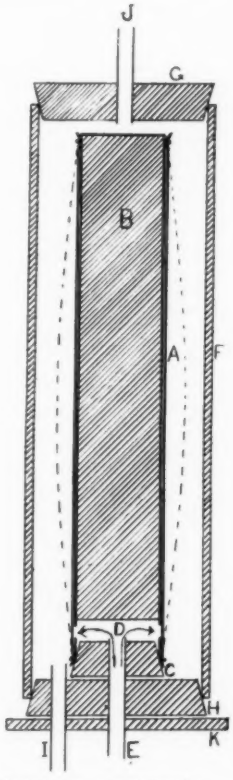


Fig. 1.—The sphygmoscope, laboratory construction. X ½. Description in text.

will be impossible to determine low diastolic pressures. The rubber tube is securely fastened to the cylinder so as to make air-tight the space between them. In order to keep down the dead space of the instrument the brass tube is filled with wax or other suitable material, leaving at one end, however, a space deep enough to take a rubber stopper, *C*, and an air chamber, *D*, 5 to 8 mm. in depth. Two holes about 3 mm. in diameter (indicated by the arrows) are drilled through the brass tube into this air space. A hole bored through the stopper, *C*, carries the communication, *E*, between this air space,

and consequently between the space under the band tubing, and the remainder of the pressure space of the sphygmomanometer. The housing, *F*, of the sphygmoscope is a glass tube (so-called 2 inch) closed above and below by thin rubber stoppers, *G* and *H*. The lower stopper is provided with two holes through which the two tubes of the sphygmoscope base pass, one, *E*, into the inner or pressure chamber, the other, *I*, into the outer or tambour space. The upper stopper has one hole, *J*, through which communication is made with the recording tambour. To substitute this sphygmoscope for the one originally a part of the sphygmomanometer it is merely necessary to remove the tambour holder and the supporting frame on which it rests. The tambour can then

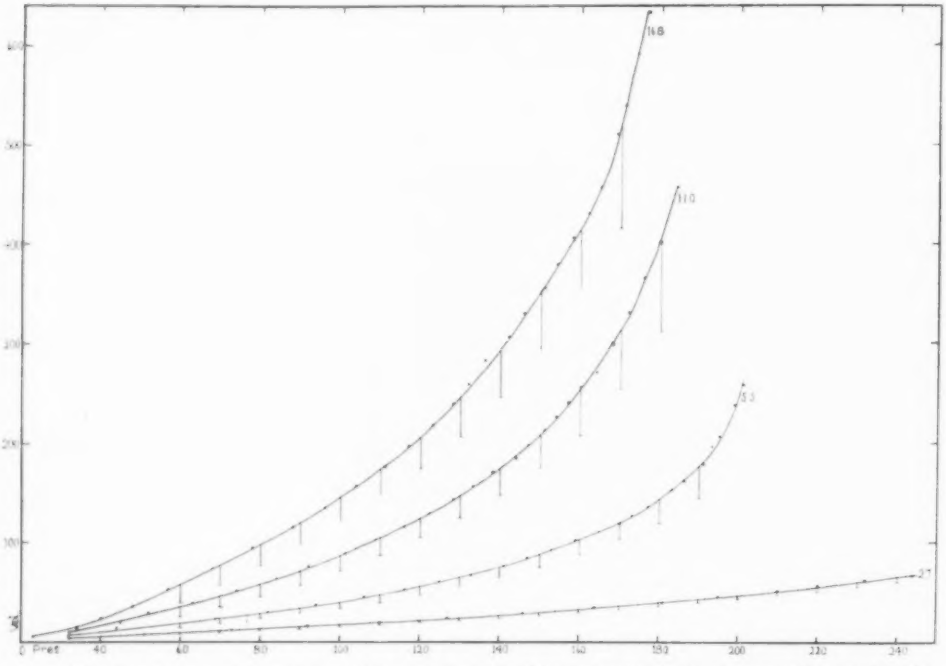


Fig. 2.—Curves obtained by plotting the extensibility (ordinates) of four different lengths (2.7, 5.5, 11.0, and 16.8 cm.) of band tubing against the distending pressure (abscissae). For further description see text.

be supported on a separate stand or on a rod which any mechanic can erect on the base of the instrument. The sensitivity (extensibility) of the sphygmoscope can be altered to suit the case under observation by varying the length of band tubing exposed to the stretching pressure. This is determined by the distance of the upper ligature securing the rubber to the brass tube, from the lower.*

Various tests have been made in order to ascertain the capabilities of this new type of sphygmoscope. In the first place the extensibility has been determined of different lengths of band tubing tied as described above to the inserted brass tube. In these experiments the pressure distending the rubber tube was increased in steps and the volume of air displaced by the ballooning tube enclosed in its glass housing was measured by the move-

*A more convenient method of accomplishing this end is described below.

ment of a bead of water occluding the lumen of a horizontal glass tube of relatively narrow bore. Preceding each increment of pressure this bead was brought back to its initial position thus eliminating inequalities in the bore of the tube as a possible source of error. The results obtained with band tubing lengths of 2.7, 5.5, 11.0 and 16.8 cm. are plotted in Fig. 2, in which the horizontal axis gives the distending pressures in mm. Hg, the vertical axis the distentions of the rubber in terms of length of the horizontal tube used to measure the volume changes.

These curves indicate, firstly, the limiting pressures tubes of various length can sustain. The shortest length, 2.7 cm., withstands a pressure of

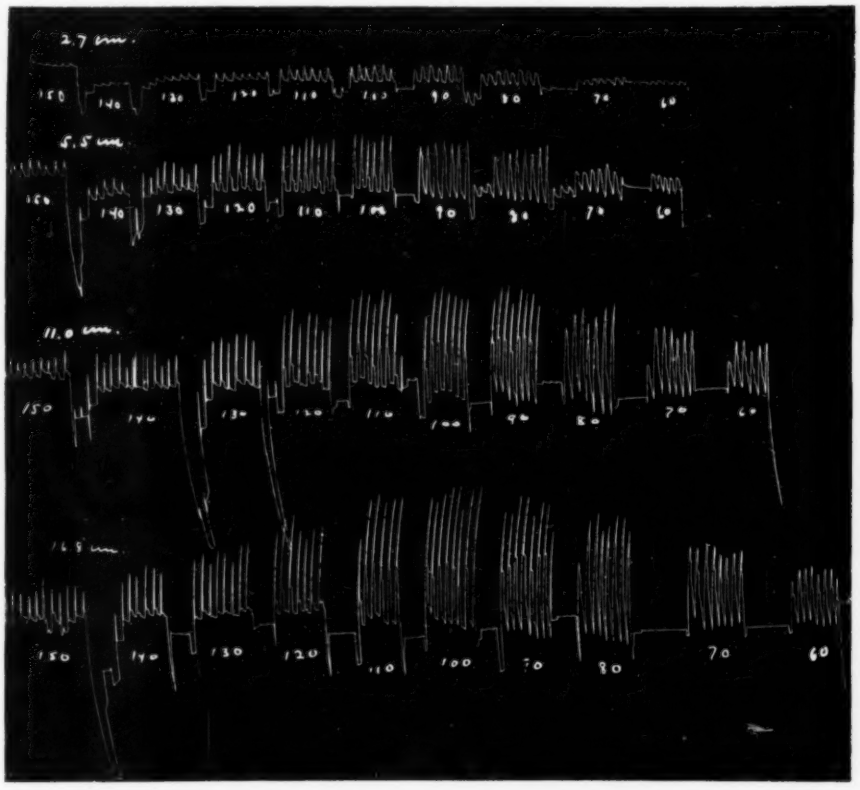


Fig. 3.—Records by the method of intermittent escapement, obtained in rapid succession from one subject, with four different lengths (the same as in Fig. 2) of rubber tube exposed, namely 2.7, 5.5, 11.0, and 16.8 cm., as indicated, but otherwise under constant conditions.

over 290 mm. Hg (the limit of the manometer employed in making the tests was 260 mm. Hg); whereas with the longest tube the limit of extensibility is rapidly approaching at a distending pressure of 174 mm. Hg. Obviously the absolute values obtained will vary somewhat according to the properties of the particular specimen and the age of the rubber.

The curves indicate, secondly, that the volume oscillations produced by a given pressure oscillation will decrease as the basal pressure decreases. This behavior is visualized in Fig. 2 by dropping from the curves at 10 mm. Hg intervals perpendiculars equal in length to the volume change produced

by the corresponding 10 mm. Hg pressure change. The inherent properties of the sphygmoscope, therefore, would tend (a) to counteract the increase in oscillation amplitude which is one of the signs of systolic compression and (b) to give a picture resembling the index to diastolic compression, namely, a decrease in the amplitude of the oscillations.

(a) Inasmuch, however, as the best index to the systolic pressure is the change in the form of the oscillations*[2, 3] the type of extensibility exhibited by the sphygmoscope does not interfere with the reading of the systolic pressure.

(b) To be in a position to estimate the degree to which the reading of the diastolic pressure might be influenced by this property of the sphygmoscope it is necessary to have clearly in mind the oscillatory sign of diastolic compression. It is not, as is usually stated in the textbooks, the last of the highest oscillations. Neither is the diastolic pressure correctly marked by the first decrease in the size of the oscillations recorded during decompression. Rather, it is the point where the accelerating decrease changes into a retarding decrease in amplitude. To state this in another way, the sign is the point of inflection of the curve of amplitude decrease.[4] This, presumably, is the same as the point selected by MacWilliam and Spencer[5]; namely, "just after the abrupt diminution" in oscillation amplitude. Now, a glance at the curves of extensibility of the band tubing (Fig. 2) shows that though the vertical lines (oscillations) decrease in amplitude with decompression, there is nowhere a point of inflection in the curve of amplitude. It is also clear that any tendency on the part of this behavior of the sphygmoscope to obscure the diastolic sign could be minimized by using a length of band tubing that yields in the region of the diastolic pressure a nearly linear curve. The curve obtained with the 2.7 cm. tube is practically linear up to pressures of 120 mm. Hg, or more, that of the 5.5 cm. tube up to 90 mm. Hg, whereas in the case of the 11 and 16.8 cm. tubes the linear ranges are considerably more limited. When we are dealing with normal diastolic pressures, therefore, the properties of the band tubing, up to lengths of 5.5 cm., would not interfere appreciably with the diastolic sign; indeed, owing to the peculiar characteristics of the sign, no confusion could result even in the case of the longest of the segments.

To put to a practical test the questions raised by these considerations the sphygmoscope employed above was attached to a sphygmomanometer and records were obtained from one and the same individual, while maintaining constant all conditions excepting the length of band tubing. The lengths tested were those used in obtaining the data for the four curves reproduced in Fig. 2. The records, shown in Fig. 3, were made by the method of intermittent escapement. It can readily be made out by simple inspection, but much more clearly by plotting the amplitude of oscillation (average height of the oscillations at each compressing pressure) against the compressing pressure (Fig. 4), that there is an inflection in the decline

*Hediger, unaware of the previous work on this subject, redescribes this change in form as a sign of systolic compression. Neither does Hediger know that instruments with which continuous decompression records of the pulse can be made, have been available for many years.

in amplitude that occurs during decompression. In three of the records this inflection (indicated by the arrows) appears between 80 and 70 mm. Hg; in the fourth record it follows 70 mm.

The systolic sign, namely, the *first* clear increase in the spread of the limbs at the base of the pulse wave[2] in each of the four records appears in the 120 mm. series of oscillations, and is perfectly definite, except in the case of the record obtained through the medium of the shortest segment (2.7 cm.), in which the oscillation amplitude is so small that details of form are obscured by the friction between the writing point and the paper. But even here the sign is not entirely effaced.

When these blood pressure records are considered in relation to the corresponding curves of extensibility of *this particular sample* of band tubing, it becomes perfectly evident that the 5.5 cm. length is adequate for the

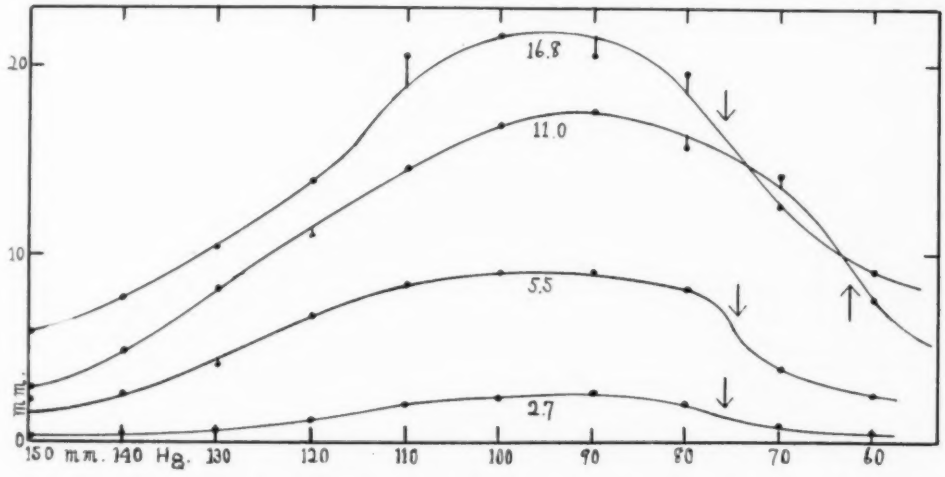


Fig. 4.—The diastolic sign in Fig. 3 elucidated by plotting the mean height of the oscillations at each compressing pressure (ordinates) against the compressing pressure (abscissae). The arrows mark the points of inflection of the amplitude decrease (the diastolic sign).

determination of normal arterial pressures. The oscillation amplitude it gives is quite sufficient; its extensibility covers a wide enough range (up to 180 or more mm. Hg); and the diastolic oscillations are written in the range (up to 90 mm. Hg); within which the extensibility curve of the rubber still is practically linear. A tube 10 to 12 cm. long, it is seen, would under normal conditions give an oscillation that is higher than is really necessary. Furthermore, the extensibility of a tube of that length increases in such a way that pressures exceeding 160 mm. Hg could not be satisfactorily measured with it. These characteristics, however, are just the ones that would facilitate the measurement of the arterial pressures in conditions of hypotension. Finally, for the determination of the arterial pressures in cases of hypertension it would be necessary to have a sphygmoscope that would record oscillations up to 250 to 300 mm. Hg; this, the shortest (2.7 cm. segment) would do. In hypertension cases, furthermore, the oscillation amplitude would probably be considerably higher than in the normal records

shown in Fig. 3 and Fig. 7; for along with the increase in arterial pressure there usually is an increase in pulse pressure also.

To record satisfactorily the oscillations transmitted by the rubber bulb sphygmoscope a compound lever was necessary; a simple lever suffices with

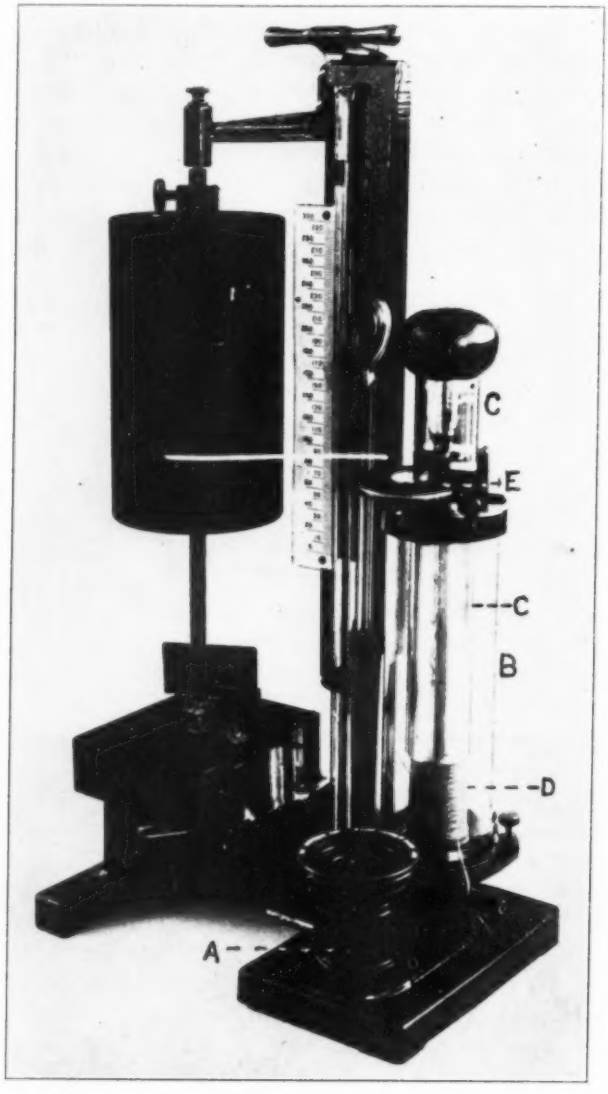


Fig. 5.—The sphygmomanometer as now constructed.
A = compound stopcock
B = sphygmoscope
C = sliding sleeve for the adjustment of the sensitivity of the sphygmoscope
D = band tubing
E = tambour, with simple lever

the present sphygmoscope, to which the records reproduced in Figs. 3 and 7 bear witness. Not only is construction thus simplified but inertia also is diminished and consequently instrumental deformation of the recorded pulses.

This sphygmoscope, together with an arm band and pump, form a convenient and accurate sphygmograph which can be used in connection with a polysphygmograph in the manner suggested by Halsey.[6]

THE SPHYGMOSCOPE ON THE MODIFIED SPHYGMOMANOMETER

The foregoing considerations and observations clearly indicate the advantages that are to be gained by adapting the new form of sphygmoscope to the sphygmomanometer.* The manner in which this has been done is indicated in Fig. 5 which pictures the entire sphygmomanometer as it is constructed in this laboratory. A detailed description of the instrument as a whole is unnecessary. Neither need a detailed description be given of the sphygmoscope since the method of putting together the simpler form, described above, suffices to indicate the construction of the shop-made form. It is necessary, however, to call attention here to the device that has been added to facilitate changing the length of band tubing exposed to the compressing pressure.

This is accomplished by a metal tube which slides over the band tubing from above and thus prevents the parts of the rubber covered by it from extending under the distending pressure. With inserted metal cylinder and band tubing exactly of the dimensions specified above, a piece of Shelby steel tubing (*C*, Fig. 5) 6 inches long, 1⅛ inches in outside diameter, and Number 16 gauge, does perfectly for this purpose. The motion of the metal sleeve over the rubber tube is facilitated by keeping the surfaces covered with powdered soapstone and by advancing the sleeve with a spiral motion. To prevent leakage in the tambour space an accurate sliding fit between the steel tube and the brass top of the sphygmoscope is necessary.

It might be added that the recording tambour now connects directly with the sphygmoscope by means of a conical joint. It is, therefore, a simple matter to remove the tambour for the purpose of stretching its head or in order to record with it the oscillations upon another than the kymograph attached to sphygmomanometer.

Advantage is taken of this opportunity to describe also certain changes in the compound stopcock which, though introduced some years ago, have never been published. These alterations have been made in order to facilitate the determination of the pressures by the method of intermittent escapement and also the adjustment of the capillary openings into the tambour and pressure spaces. Fig. 6 gives the plan of the new stopcock. The designations are now on a dial attached to the plug of the stopcock and are *IN*, *CLOSED*, *INT*, *SLOW*, *CLOSED*, *FAST* and *OUT*. As heretofore, at position *IN* the pressure space can be inflated while the tambour head is protected by an opening connecting the tambour space freely with the exterior. At *CLOSED* the tambour and pressure spaces both are closed with the exception of a very minute leak in the former (now adjustable by means of a knurled screw under the tambour) provided in order to eliminate the effects

*The parts ready for attachment to instruments obtained in recent years from Schneider Brothers, as well as the sphygmomanometer as a whole, can be supplied by Mr. John H. Zimmer, Mechanical Department, Washington University School of Medicine, to whom the authors are indebted for valuable assistance in working out many of the mechanical details.

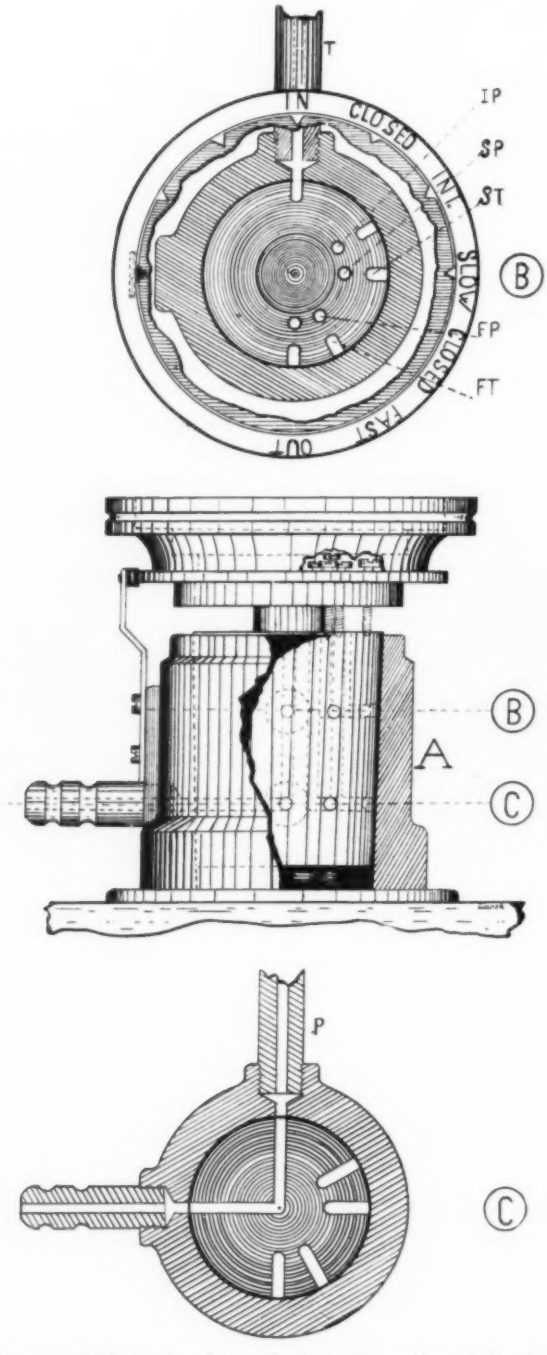


Fig. 6.—*The compound stopcock. A*, front view; *B*, section at the level of the tube, *T*, connecting with tambour space; *C*, section at the level of the tube, *P*, connecting with the pressure space. The designation that is aligned for reading from the front of the instrument (like *IN*, in the figure) indicates the operating position of the stopcock. The openings through the top of the plug are arranged on two circles, those on the outer circle connecting with the tambour space through the upper tube, *T*, those on the inner circle with the pressure space through the lower tube, *P*; of these *IP*, *SP*, *ST*, *FP*, and *FT* are adjustable by means of the screws shown in *A*. The openings operative in any given procedure are on the radius passing through the corresponding designation.

of venous engorgement and muscular movement on the general level of the lever. At *INT* (intermittent escapement) the compression diminishes at a rapid rate, controlled by the screw over the opening, *IP*, and at the same time the tambour space is in free communication with the exterior; and when the cock is turned back to *CLOSED* the instrument records the oscillations under the new compressing pressure thus obtained. By dropping the compressing pressure in this way in steps of 5 or 10 mm. Hg determination can readily be made of the arterial pressures by the method of intermittent escapement (see Fig. 3).

The remaining positions of the stopcock accomplish what was done by the whole of the cock originally. At *SLOW* the air escapes slowly from the pressure space (through *SP*), and at the same time undue sinking of the tambour head is obviated by an adjustable leak, *ST*. The arrangements are the same as at *FAST* except that here the adjustments (by the screws over *FP* and *FT*) should be such as to permit of a somewhat more rapid fall of the compressing pressure. At *OUT* the tambour space and the pressure space both open freely to the exterior. The rates of air escape from the pressure space

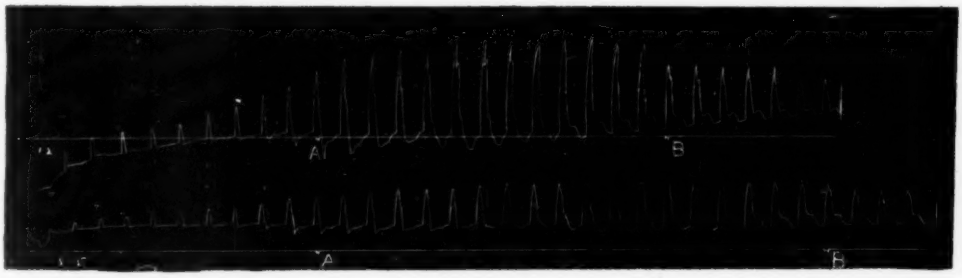


Fig. 7.—Two records made in succession from the same subject by the method of continuous escapement, the upper with 12 cm. of band tubing exposed, the lower with 5.5 cm. The marker signalling the sounds writes 2 mm. ahead of the pulse marker in the upper, and 3 mm. ahead in the lower record. X½.

and the rates of entrance of air into the tambour space are determined by adjustment of the screws in the stopcock all of which are now plainly in view and accessible from above (see Fig. 5).

In Fig. 7 are recorded two determinations made by the method of continuous escapement with the new apparatus. Both tracings were obtained from the same subject, the upper with the sliding sleeve withdrawn so as to expose 12 cm. of the band tubing in the sphygmoscope, the lower with 5.5 cm. of the band tubing in use. The advent of the first Korotkoff (pistol shot) sound and the change of the sounds from those of the third to those of the fourth phase (dulling) were objectively signalled while making these records. The marks, *A*, signalling the advent of sound, fall, as can be seen, 1 to 2 pulses later than the graphic sign (the first change in the form of the pulse). And the marks, *B*, signalling the dulling of the sounds, fall precisely at the point where the accelerating decline in pulse amplitude changes to a retarding decline.

SUMMARY

A sphygmoscope with adjustable sensitivity is described which is a suitable substitute for the rubber bulb sphygmoscope used in recording the pulse, especially in connection with the recording sphygmomanometer, and tests of its capabilities are supplied.

Changes in the recording sphygmomanometer making for improved legibility of the blood pressure signs and for greater ease in the manipulation of the apparatus are reported. These consist principally in the substitution of a simple for the compound lever, and in moving the adjusting screws from the bottom to the top of the stopcock into plain view.

REFERENCES

[1]Erlanger, J.: Johns Hopkins Hos. Rep., 1904, xii, 53.
[2]Erlanger, J.: Am. Jour. Physiol., 1908, xxi, xxiv.
[3]Hediger, S.: Deutsch. Arch. f. klin. Med., 1922, cxli, 117.
[4]Erlanger, J.: Am. Jour. Physiol., 1921, lv, 84.
[5]MacWilliam, J. A., and Melvin, G. Spencer: Heart, 1914, v, 153.
[6]Halsey, R. H.: Arch. Int. Med., 1916, xvii, 540.

RAPID-MIXING FLASKS FOR THE PREPARATION OF THE STANDARD ANTIGEN DILUTION USED IN THE KAHN PRECIPITATION TEST*

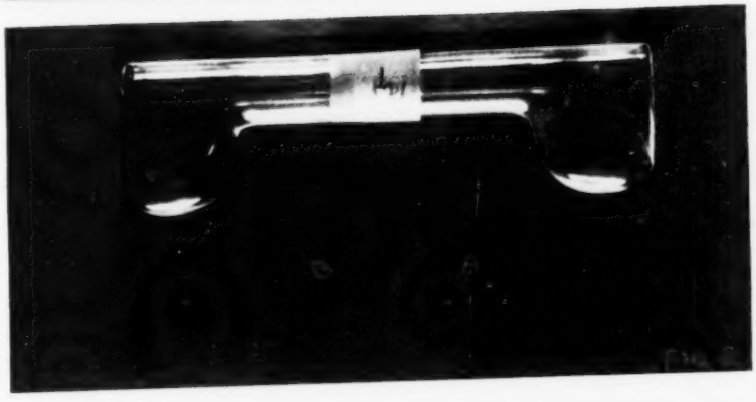
By Thomas J. Porro, Tacoma, Wash.

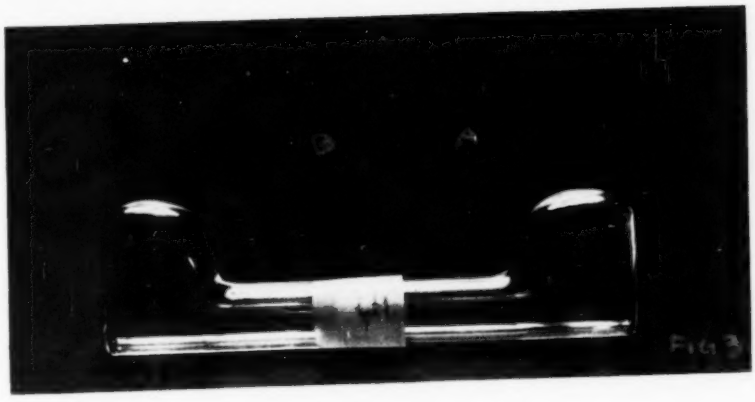
THE test tubes in general use for the preparation of the standard antigen dilution used in the Kahn test, while quite satisfactory, require rapid manipulation with some loss of the mixture, down the sides of the tubes or by accidental spilling.

Fig. 1 shows one flask ready to receive antigen and another for saline. The bulbs blown on the sides keep the saline and antigen separate while the flasks are being joined together (Fig. 2). The flasks are then turned with the bulbs up (Fig. 3), giving the liquids a smooth surface for flow from one into the other, with rocking. After mixing, the antigen dilution is drained into flask *B*. The large area in the bottom permits shaking of the contents, without spilling, after the usual lapse of time.

*From the Serological Department of the Porro Biological Laboratories, Tacoma, Wash. These flasks may be procured from Porro Biological Laboratories, Tacoma, Wash.
Received for publication, June 9, 1926.

A
B





A CONVENIENT METHOD OF COLLECTING SMALL AMOUNTS OF SERUM*

By Max Shaweker, M.D., Dover, Ohio

IT FREQUENTLY occurs in the collection of small amounts of serum, such as complement serum from the guinea pig, that a few red blood cells wash away from the clot and interfere with pipetting off the serum without considerable waste. A method which we use, easily removes this objection and gives the maximum yield of serum from a given amount of blood. The process consists of collecting blood from cardiac puncture, carotid section, venipuncture or other method and placing it in Petri dishes at room temperature for a half hour. About 5 to 10 c.c. is the optimum amount of blood per standard 100 mm. dish. The dish is slanted slightly so that the blood will not completely

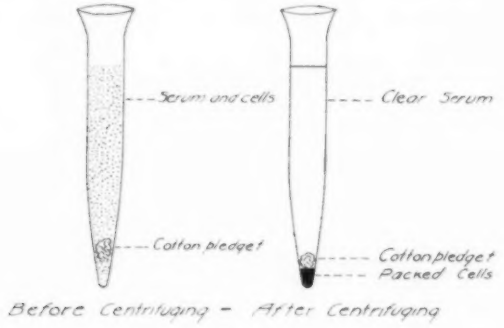


Fig. 1.

cover the bottom of the dish, leaving a small area in which to collect and pour off the serum. After the clot is well organized it is cut with a needle or knife in small lines radiating to the bare spot in the Petri dish. The serum then gravitates to the bare side of the dish taking perhaps an hour or even overnight, as in guinea pig complement collection. Into a dry 15 c.c. centrifuge tube is placed a small piece of absorbent cotton. A little shaking may be required to throw it to the bottom of the tube. The serum from the Petri dishes with any reasonable amount of red blood cells which loosen from the clot is now poured into the centrifuge tube. After a brisk centrifuging, the cells will gravitate through the cotton and pack below it. Then the tube can be completely inverted and the serum decanted without disturbing the cells packed under the cotton pledget. Serum so collected is entirely free of turbidity due to accidental admixture of red blood cells and is usually hemoglobin stained.

*Received for publication, June 11, 1926.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Pack, G. T.: The Pathology of Burns. Arch. Path. and Lab. Med., May, 1926, i, No. 5, 767.

Various etiologic agents may be responsible for causing burns and scalds: dry heat, moist heat of various kinds, the actual flame, heated solid bodies, electricity, roentgen rays, radium, sunlight and caustic chemicals.

Thermal traumas are best classified in six degrees to denote the various depths of tissue invaded or destroyed. The local tissue changes progress through the various stages of destruction or burning, inflammation and sloughing and finally regeneration and repair. The amount of local tissue destruction varies from simple erythema and the degrees of vesication to an involvement of the entire epidermis, dermis, subcutaneous tissues and even muscle and bone, when there is great intensity or prolonged contact of the heat. Scarring is inevitable when the papillary layer of the skin is destroyed.

The liver, brain, bone marrow, and kidneys of the burned patient may exhibit hyperemia, focal necroses and parenchymatous degenerative lesions. The suprarenal glands are swollen and deep red owing to hyperemia and ecchymotic areas of hemorrhage among the parenchymal cells. The spleen, the lymph glands and the solitary and agminated lymph nodules of the intestinal tract are the seats of toxic focal necroses occurring in the centers of the germinal follicles. This necrosis is quickly followed by the rapid proliferation of endothelial leucocytes.

The erythrocytes undergo certain alterations in structure and disturbances of function. Leucopoiesis is stimulated by the burn toxin, so that leucocytosis occurs with an increased percentage of neutrophilic polymorphonuclears. A predisposition to thrombosis exists because of the leucocytic disintegration, the venous stasis and the viscidity of the concentrated blood. A goodly portion of the visceral pathology has been attributed to the presence of minute capillary thrombi. The rapid and continuous loss of fluid from the blood in burned patients quickly induces a marked concentration of the blood. This becomes a factor of the greatest importance in the development of the syndrome characteristic of burns, and a factor of prime significance in the fate of the person concerned. Changes observable in the chemical composition of the blood during burns vary at most only slightly from the normal limits.

The urine is subjected to certain quantitative and qualitative alterations, such as oliguria, albuminuria, albumosuria, and acetonuria.

The hypothetic burn toxin has its source of origin in the burned tissues, from whence it is absorbed and circulates in the blood, being carried by the red blood corpuscles. The exact nature of the burn toxin is as yet unknown, but it is probably closely related to the primary and secondary proteoses or to other products of protein disintegration.

The most common complication of burns and scalds is a secondary pyogenic infection of the burned area. Other complications which occasionally occur are: nephritis, septicemia, tetanus, wound hemorrhage, meningitis, apoplexy, pneumonia, amyloid infiltration of the viscera and cicatricial contractural deformities. The inconstant duodenal ulcer of burns is due either to the irritant action of the bile, which owes its injurious ability to its content of the presumptive burn toxin, or to the production of infarction of the duodenal mucosa by septic emboli.

Electric burns occur at the areas of greatest resistance to the current, particularly at the points of entrance and exit. The electric burn is characterized by extensive sloughing of tissue, delayed healing and the absence of vesicles. The type of lesion produced by lightning varies according to whether the mechanical or thermal action of the electricity pre-

dominates. Lightning burns are further characterized by the occurrence of peculiar pink or lilac colored arborescent skin markings.

The tissue degeneration caused by the roentgen rays and radium is due to one or both of two mechanisms; either the changes are wrought by the direct action of the rays per se, or the localized necrosis and gangrene is the result of ischemic phenomena produced by sclerosis of the blood vessels supplying the skin. These burns are slow to appear, slower to heal and have a decided tendency to undergo malignant degeneration.

Sunburn is an erythematous or erythematovesciular burn produced by the action on the skin of the ultraviolet components of the complex solar rays.

Chemical causties produce a lesion which exhibits a central solution, a deeper layer of cell death and necrosis and a peripheral layer in which the inflammatory reaction appears. Caustic alkalis penetrate deeply and bring about their pathologic action by three different means: they saponify fats; they withdraw water from the cells; they form alkaline albuminates. The concentrated mineral acids likewise withdraw water from the cells; they dissolve epithelium and connective tissue; they form acid albuminates and acid metaproteins. The corrosive action of the organic acids is proportional to their volatility. The character of the burns of the alimentary tract depends on the nature of the caustic, its concentration, the amount swallowed, the duration of contact and the size of surface involvement. Caustic burns of the eyes are exquisitely painful and are often followed by visual impairment secondary to iridochoroiditis, corneal opacification, etc.

Esquier, G.: Bacteriologic Examination of the Urine for Koch's Bacillus. Paris Med., Nov. 14, 1925, xv, 405.

The specimen should be fresh.

If the amount of urine is sufficient one or two centrifuge tubes with a capacity of 12 c.c. are filled, otherwise whatever amount of urine is received is centrifuged. The specimens are centrifuged for from five to ten minutes. The supernatant liquid is saved for inoculation purposes.

The sediment is stirred with a pipette so as to thoroughly mix the different layers, then a part of it is aspirated and is used to make three smears.

The first smear is colored with ordinary methylene blue or the stain may be diluted to 1:10 or 1:20. The second method is stained by Gram's method so as to obtain some information as to the presence of gonococci or colon bacilli.

The third smear is used for examination for the tubercle bacillus.

The following technic is used:

1. Fixation by heat.
2. Immersion in Ziehl's carbolated fuchsin.

Fuchsin	1.
Pure crystallized phenol	5.
Absolute alcohol	10.
Distilled water	100.

Triturate the fuchsin and alcohol in a small mortar, add the phenol; add two-thirds of the water in small portions, emptying the mortar after each addition, then rinse the mortar with the remaining water and mix all together. Allow to stand for twenty-four hours and then filter.

Smears should be stained for from fifteen to twenty hours; usually overnight will suffice. An immersion of forty-eight hours will not alter the smears.

3. Rinse in water.
4. Decolorize with nitric acid (1:3), 5 minutes.
5. Rinse in water.
6. Decolorize with 95 per cent alcohol, 5 minutes.
7. Rinse in water.
8. Counterstain with methylene blue.
9. Wash and dry.
10. Examine with the oil immersion objective.

By this procedure the tubercle bacilli are almost always found. They are usually granular and arranged in masses. Sometimes they are isolated and some are found in contact with polymorphonuclears. There may be only a very few but with a good preparation and with a properly lighted microscope they can be easily seen.

When this procedure is used there is no danger of mistaking smegma bacillus for the tubercle bacillus as all the bacteria seen are tubercle bacilli.

By this method positive results may be expected in 88 per cent of cases.

Reiter, H.: The Cultivation of Pure Cultures of Spirocheta Pallida, Spirocheta Dentium, and Spirocheta Recurrentis. Klin. Wchn., March 12, 1926, v, 444.

The best medium for Sp. pallida and Sp. dentium is a mixture of equal parts of horse-serum and normal saline with sterile pieces of rabbit or guinea pig kidney or liver.

The following are useful liquid media:

A. Liquid medium for Spirocheta pallida.

1. Normal rabbit serum is mixed with a 1 per cent normosal solution and pieces of the brain of these animals are inserted. This mixture is kept for twenty-four hours at a temperature of 56° C. Its sterility is controlled for twenty-four hours at a temperature of 37° C.

The inoculation is performed with capillaries. Sterile paraffin oil or white vaseline is used for the covering. The incubation is made at 37° C.

The optimum of the growth is on the fourth or fifth day. The cultures remain transparent and odorless. The reinoculation is made on the seventh day.

2. Human or cattle liver is kept for forty-eight hours at a temperature of 37° C. The secreted fluid is filtered and its sterility is controlled. This autolysate is mixed in equal parts with a 1 per cent normosal, ascites (autolysate-normosal-ascites).

The inoculation, covering and incubation are the same as in 1.

The optimum is on the second day. There is no macroscopic change in the medium and no odor. The reinoculation is made on the fourth day.

B. Liquid medium for Spirocheta dentium.

1. Normal mutton serum is mixed with a 1 per cent normosal solution and pieces of rabbit or guinea pig brain are inserted. Heating and sterility test as in 1. *A.*

The inoculation, covering and incubation are the same as in 1. *A.*

The optimum is on the fourth or fifth day.

The smell is typical for Sp. dentium; the macroscopic opacity is seen to settle in the form of a greyish-white cloud. The reinoculation is made on the seventh day.

2. This is the same procedure, only replacing mutton-serum by rabbit-serum. The growth is slower, the optimum is on the fifth or sixth day. The smell is typical but there is no sedimentation.

C. Liquid medium for Sp. recurrentis.

The medium is the same as for Sp. pallida, but the addition of a quantity of fresh sterile guinea pig blood amounting to 10 per cent of the total is necessary.

The inoculations, covering and incubation are the same as in 1. *A.*

The optimum is on the fifth day. There are no macroscopic changes. The reinoculation is made on the sixth day.

Minot, G. R., and Isaacs, R.: Lymphoblastoma (Malignant Lymphoma), Age and Sex Incidence, Duration of Disease, and the Effect of Roentgen-ray and Radium Irradiation and Surgery. Jour. Am. Med. Assn., April 17, 1926, lxxxvi, 1185; April 24, 1926, lxxxvi, 1265.

Four hundred and seventy-seven cases of all types of lymphoblastoma, excluding lymphatic leucemia, have been studied. Four hundred and one of the patients are dead; 238 were treated by roentgen-ray or radium irradiations, and 163 were not. Sixty-eight per cent of all were males.

The ages at which lymphoblastoma most frequently began were from 20 to 24, when most of the cases were of the Hodgkin type. The next ages when persons were most susceptible were from 35 to 39. At this time in life other forms of lymphoblastoma than

Hodgkin's disease composed the majority of cases. Females tended to have the different forms later in life than males.

At all ages except at the age of puberty the number of males exceeded the females, and a relatively large percentage of women had the condition at or near the time of the menopause.

The duration of lymphoblastoma in the females was apt to be longer than in the males. It tended to be longer in men over 35 years old and in women under 25, than in younger males and older females, except when the latter were from 33 to 44 years of age.

Comparisons between the lengths of the course of disease in irradiated and nonirradiated patients do not indicate that such treatment significantly affects the duration of lymphoblastoma. It may do so in selected cases and yet can be blamed, in cases of short duration in the patients that were below the age of 24, for the greater incidence of those in which irradiation was given than those in which it was not.

The chances of lymphoblastoma lasting long were relatively great for those first irradiated when their disease was in an early stage. However, the duration of any such case can be paralleled by one given no special form of therapy.

Surgery probably can influence beneficially the duration of some cases of lymphoblastoma, particularly if it is employed early and thoroughly and is followed by irradiation.

The type of case usually treated early by surgery or by irradiation is apt to be the same as that destined by nature to last a long time.

The average duration of lymphoblastoma in the 401 deceased patients was 2.76 years, yet about 10 per cent of both the irradiated and nonirradiated had the disease for six or more years. A greater percentage of the seventy-six living patients have had lymphoblastoma this long duration of time. Although among them the frequency of surgical and early irradiation treatment is greater than for the deceased group, the treatment by modern methods will explain at the best but partially the increased percentage of cases of long duration.

Irradiation is undoubtedly of great value to patients with lymphoblastoma; it alleviates symptoms, decreases the size of lesions, and improves the patients' efficiency, in spite of the fact that it does not appear to influence importantly the duration of the disease.

Mann, F. C., and Bollman, J. L.: Liver Functional Tests. Arch. Path. and Lab. Med., May, 1926, i, No. 5, 682.

The most important of the tests suggested for measuring hepatic function have been studied in normal animals, animals with an Eck fistula, animals with permanently reduced amounts of hepatic tissue, and animals with the liver completely removed. As a result of these observations, a few positive statements can be made relative to the functional capacity of the liver, a few suggestive signs of hepatic deficiency can be presented, and the tests of hepatic function suggested can be evaluated by means of a standard technic.

The bilirubinemia which follows removal of the greater portion of the liver is but transitory, and the remaining portion of the liver is soon able to excrete all of the bile pigment formed in the animal. Partial removal of the liver has little demonstrable effect on the rate of disappearance of dyes injected into the blood unless the amount of dye injected is excessive, although complete retention of the dye is easily demonstrated in the completely dehepatized animal. The carbohydrate metabolism of animals with greatly reduced amounts of hepatic tissue, so far as tests of hepatic function are an indication, is also maintained at an approximately normal level, and only slight differences from normal may be found in the amount of sugar in the blood. The use of glucose or levulose tolerance tests also fails to bring out any marked deviation of these animals from normal. The marked changes in the formation of urea, accumulation of amino-acid and excretion of ammonia, which are specific in the dehepatized animal, are difficult to demonstrate in the animal with greatly reduced hepatic tissue. The decrease in the destruction of uric acid, however, may be demonstrated in animals with reduced hepatic tissue. Physiologic reactions following the administration of certain toxic substances may indicate that the reduction in the amount of hepatic tissue reduces the animal's tolerance to these substances, but the chemical tests employed do not show any extensive decrease in the rate of conjugation of these substances.

In summarizing the results of the experiments, as they would appear to apply to the clinical tests of hepatic function, the following can be suggested: There appears to be no physiologic basis for many of the tests employed to measure the functional capacity of the liver. So far as carefully controlled experimental data may apply to such problems, most of the tests should be discarded. Some of these tests which could not be proved experimentally to have any value in a known and controlled condition of hepatic deficiency may be of value clinically, either because they are an index of disease, not necessarily wholly hepatic, or because spontaneous disease of the liver may affect the function differently from experimental procedures, or because man may be somewhat different from the dog. However, the value of such tests should be accepted only with data obtained in cases in which the hepatic disease is proved either by a definite clinical diagnosis or anatomically at operation or necropsy.

The tests which would appear from these experimental data to have value in relation to estimation of the function of the liver are as follows: The van den Bergh tests for bilirubin in the blood should be of value as a measure of a condition in which the liver is either directly or indirectly, but not necessarily predominantly, affected. The physiologic basis for the use of the dyes which have been employed as tests of hepatic function is the fact that they appear to be excreted mainly by the liver. Experimentally the hepatic function could not be sufficiently diminished to show a definite relation of hepatic insufficiency to retention of dye. Since, however, there is a definite retention of the dye in certain cases in man, this would appear to be one of those tests whose value can be determined only by its careful and controlled use clinically, as has been done by Rowntree and his associates. There is a definite experimental basis for the elaboration of a test of glycogen mobilization which might bear the same relation to the activity of the liver in relation to carbohydrate metabolism as the quantitative bilirubin test does to pigment metabolism.

The best test of functional deficiency of the liver in the dog which has been found is based on the facts that destruction of uric acid depends on the liver, that this is the most easily injured of the known functions of the liver, and that the amount of uric acid excreted in the urine appears to bear a definite relation to hepatic damage. This test is easily performed by determining the amount of uric acid eliminated in the urine during a standard period of time following the ingestion of a standard meal with high content of purin. Whether or not such a test will be of value in man remains to be proved.

Plaut, A.: Bilharzia Infection in an Apparently Normal Appendix. Arch. Path. and Lab. Med., May, 1926, i, No. 5, 712.

Report, illustrated with eleven microphotographs, of a case in which ova of Schistosoma hematobium were found in sections of an apparently normal appendix removed in the course of an operation for retroversion. Ova were later found in the feces and in sections from the cervix.

Weidman, F. D., and Sunderman, F. W.: Hypercholesterolemia. The Normal Blood Cholesterol Figures for Man and the Lower Animals. Arch. Dermat. and Syph., November, 1925, xii, 679.

A review of the findings of various workers using a variety of methods: gravimetric, colorimetric, and spectrometric, and of the authors' results with the Myers-Wardell technic as modified by Karr and Oser.

The normal cholesterol values were determined for four men, three monkeys, four dogs, four cats, four rabbits and three guinea pigs, each being tested on four or five occasions.

The average normal values obtained were:

Man	140–160 mg. per cent.
Monkey	121–136 mg. per cent.
Dog	111–118 mg. per cent.
Cat	94–100 mg. per cent.
Guinea pig	81– 87 mg. per cent.
Rabbit	64– 80 mg. per cent.

Figures are also quoted from the literature for various other animals.

As far as can be told at present the normal values for man lie between 160 and 180 mg., though influenced by diet and the method employed.

In animals there is no apparent relation between the diet and the blood cholesterol.

There are so many possibilities of error in current colorimetric methods that each worker should first run normal controls for himself when dealing with animals.

Zamorani, V.: Bilirubin in Feces and Meconium of Nursing Infants. Clin. Pediatr., 1925, xxiii, 9.

The feces of nursing infants contain unaltered bilirubin; the feces of children over one year of age contain only urobilin and urobilinogen.

Bile secretion begins in the sixth fetal month and biliverdin appears in the meconium. In the first eight days of life the bilirubin excretion is about 0.13 per cent of the feces, decreasing to 0.01 per cent after six months.

Meulengracht, E., and Iversen, P.: Blood Sugar in Pernicious Anemia. Deutsch. klin. Med., 1925, cxlviii, 1.

In a series of 250 examinations a moderate increase in blood sugar was noted in pernicious anemia. There was no apparent relation between blood sugar and the hemoglobin content.

During acute stages the blood-sugar curve may be raised or lengthened due, not to the anemia, but to the accompanying intoxication.

Bueltemann, H.: Lange and Huer's Photochemical Test of Serum Applied in Gynecology. Münch. med. Wchn., Feb. 5, 1926, lxxiii, 238.

To 0.1 c.c. of fresh serum add 0.2 c.c. of 0.1 per cent silver nitrate solution.

The reaction is read as negative (no sediment, supernatant fluid dark brown), or positive (dark brown sediment and limpid supernatant fluid), the positive reactions being graded II, III, and IV in accordance with the amount of sediment and the clarity of the supernatant fluid.

Bueltemann found positive reactions in carcinoma, operable cases giving II and III reactions, inoperable, IV.

In pregnancy, as well as recent and uncomplicated abortion, the reaction was consistently negative.

After delivery and all during lactation the reaction was positive. In febrile conditions occurring during pregnancy or in any febrile reaction the test was positive.

The reaction is held to result from a displacement of the proportional protein-globulin content of the serum.

Bassler, A.: A Quantitative Test of Digestive Pancreatic Activity Easily Applied Clinically. Arch. Int. Med., February, 1925, xxxv, 162.

Technic of test.—The reagents are: 1. A standard starch P_H 6.7 sodium chlorid solution made up as follows:

To solution *A*, when cool, 50 c.c. of solution *B* (plain water acts quite as well), and 25 c.c. of solution *C* and distilled water should be added to bring the volume of the mixture to exactly 400 c.c. This should be freshly prepared.

Solution *A*. In a beaker, 2 gm. of the starch to 100 c.c. of cold distilled water should be mixed thoroughly and then heated. Under constant stirring, this should be brought to boiling and then cooled.

Solution *B* is a standard buffer solution P_H 6.7; 50 c.c. fifth molar acid potassium phosphate and 21 c.c. fifth molar sodium hydroxide solution are accurately delivered in a 200 c.c. volumetric flask, and the contents brought to the exact volume with distilled water.

Solution *C* is a 1 per cent sodium chlorid solution.

2. A twenty-fifth normal iodine solution, with a dropper cork in the bottle.

The reagent solution should be ready so that no time is lost in carrying out the test, because pancreatic solution is stated to lose its diastatic efficiency at room temperature at the rate of about 25 per cent in the first twenty minutes after extraction, and 70 per cent in three hours.

The procedure is as follows:

In each of ten tubes (6 by 0.5 inches, 15.2 by 1.2 cm.) in a rack and numbered from 10 to 1, one should accurately pipette with a 1 c.c. pipette marked in hundredths, fraction .1 of the duodenal return, water and reagent as shown in the accompanying table.

PROCEDURE OF TEXT

Number of tube	10	9	8	7	6	5	4	3	2	1
Duodenal return, c.c.	0.1	0.11	0.125	0.14	0.17	0.20	0.25	0.33	0.5	1.0
Water, c.c.	0.9	0.89	0.875	0.86	0.83	0.80	0.75	0.67	0.5	0.0
Reagent, c.c.	4	4	4	4	4	4	4	4	4	4
Units (author's) per 100 c.c.	20	18	16	14	12	10	8	6	4	2

When set up, shaken and ready, the rack is put in an incubator at 38° C. or a water-bath for thirty minutes. The rack of tubes is then taken out: the contents of each tube is shaken; a drop of the twenty-fifth normal solution of iodine is added to each tube beginning with Tube 1; twenty minutes time is allowed for the changing of colors to become settled, and the estimation of units is made according to the furthest tube to the left that is achromic. The result is then expressed in units of pancreatic enzyme efficiency. No more than one drop of iodine solution should be added because the more the iodine, the deeper the colors, the less definite the comparison and the less accurate the test.

Ordinarily, the tubes having the largest amounts of duodenal return will be green. This shades down from Tube 1, becoming lighter and fainter to one having a colorless content. The achromic tube represents the amount of return from the duodenum that contains one-fiftieth unit of amylase. Generally the tube to the left of this tube shows a light pink or gray and those more toward Tube 10 shade deeper into reddish purple. At the achromic zone, should the right tube be a sage green and the one to the left of it a very light pink, the achromic point would be between these two tubes. For instance, if Tube 5 were green and Tube 6 pink, the units per hundred cubic centimeters would be eleven. The tube that contains the one-fiftieth unit is divided by fifty, which gives the units per cubic centimeter. The calculation used is:

Units of amylase in 1 c.c. duodenal return = units amylase (pancreatic efficiency) per hundred cubic centimeters duodenal return.

For instance, in a test that shows achromic in the sixth tube:

$$
\begin{array}{r}
.12 \\
50/6.00 \\
50 \\
\hline
100 \\
100 \\
\hline
\end{array}
$$

$0.12 \times 100 =$ pancreatic efficiency of 12 units (author's)

It will be noted that the test is so arranged that the result in units is double the number of the tube in which the reaction occurs; thus the sixth tube equals twelve units.

The test, as described, takes care of most instances of low pancreatic activity, and meets clinical requirements in a routine way. Should no achromic point be present in the first tube, a greater amount of duodenal return may be used, and, according to the amount to arrive at the achromic point, a calculation may be made.

The test is so arranged that the extreme normal ranges occur between the fourth and the seventh tubes (from eight to fourteen units), giving sufficient tubes for errors of the gland on each side of these. The average units in normal persons are between ten and twelve, and, as experience multiplies, we find six units is suspiciously low (slight hypopancreorrhea). Up to the present, in even late carcinoma, marked fibrosis, or acute and sup-

purative pancreatitis, the test, as outlined, answered for the low unit readings. In very active secretion, the dilution may be increased and calculated accordingly. The various contents should be added to the tubes, beginning with Tube 10 on the left and working to Tube 1 on the right. This rule should be carried out to the end of the test. This preserves the proper intervals of time in the various additions. Usually, not more than ten minutes are necessary to prepare the tubes in the rack, and this need not be figured off the incubator time (thirty minutes).

Admixture Factor to Foods.—It sometimes is desirable to learn what the admixture factor to foods is in the duodenum as a part of the test. A pancreatic juice may contain a greater or lesser amount of enzyme index than normal; it may be hyperfluid or diminished, and stenosis of the duct or at the ampulla may exist. In the test outlined in the above, a juice may very occasionally show a low enzyme index in a unit of return, because the return is watery compared to normal, or it may be too high because of concentration. In abnormal findings of the test described, it is well at times to find out what the admixture factor is; and, while the procedure about to be described has an element of error, it is still accurate enough for clinical purposes. For this test, when the tip of the tube is in the duodenum, the patient drinks the following mixture through a glass tube and lies on the right side. Two hundred c.c. of a 5 per cent starch solution is made up in the usual way; when cooling, 1.8 gm. of sublimed sulphur is mixed with it in a shake flask (Erlenmeyer), some large faceted beads being used to help in making the mixture uniform. This mixture will hold the sulphur in uniform suspension up to the end of the test. Having quite a flat taste, the mixture may be flavored with a few grains of saccharin and vanillin crystals.

Two Hopkins vaccine tubes are used in the test. Tube A contains part of the original starch mixture and is used as a control. Tube B contains the return from the duodenum. Each tube is filled accurately to the 5 c.c. mark and centrifugated for about two minutes at high speed. A wire with a slightly bobbed end is run down through the sulphur crystals to rearrange them, and free any bubbles or clots of starch that may have gotten in the small end. They are then centrifugated again for a minute to two, the crystals rearranged as before, and, finally, centrifugated for two minutes more. The difference between the sulphur level in the two tubes gives the admixture percentage.

Example: Tube A, 5.1 spaces; Tube B, 2.5 spaces

$$
\begin{array}{r}
.49 \\
5.1\overline{)2.500} \\
2\ 04 \\
\hline
460 \\
459 \\
\hline
\end{array}
$$

100 per cent − 49 per cent = 51 per cent admixture of duodenal contents.

It must be remembered here that water and starch do not stimulate bile flow, and such flow that may be in a return is trivial. Often, when conducting this test and bile is flowing into the duodenum, as promptly as the starch solution is given by mouth, it is checked. It is better to conduct the admixture test on a subsequent day to the one for enzyme. Obviously, with an enzyme result, and a higher or lower than normal admixture result, the unit reading of enzymatic power would have to be expressed in terms of admixture, almost all of which is pancreatic juice. The range of normal for admixture is from 40 to 80 per cent. with an average normal of 58 per cent in several hundred cases. The lengths of time for the starch to appear in the bottle is from five to fourteen minutes, with an average of six minutes, and the time for sufficient return for the tubes runs from three to twenty-five minutes, with an average of fourteen minutes. This test may be employed to diagnose stasis or obstruction in the duct of Wirsung or at the ampulla (diminished quantity of pancreatic juice with normal enzyme activity). This test is only approximately accurate.

Stasis and Obstruction.—The patient is prepared as in the foregoing tests with the tip of the tube in the duodenum. Then about 100 c.c. of olive oil is drunk, and the patient lies on the right side. In a few minutes, a return is obtained, and 10 c.c. of this is centrifugated in a graduated centrifuge tube. The percentage of duodenal secretion in this test, almost all of which is bile, in the extreme ranges met with were 59 to 99 per cent, 84 per

cent being the average. The lower the percentage of admixture the surer we may be of the existence of stasis or obstruction in the bile passages. The bile and oil return appears from two to twenty-five minutes (average nine minutes), and a sufficient amount for the test in from seven to thirty minutes (average fourteen minutes).

The difference between the admixture result in the starch test for pancreatic juice and the oil test for bile represents bile flow, which in the normal is approximately 35 per cent higher in the second; this 35 per cent runs true in the low admixtures as well as in the high. Anything below 35 per cent means either excess pancreatic secretion, too low bile secretion, or delivery (biliary stasis). This test is only approximately accurate.

Hardesty, W. L.: Flocculation in Tuberculosis. Minnesota Med., March, 1926, 119.

Into four test tubes place 0.4, 0.3, 0.2, and 0.1 c.c. of serum which must be clear, not colored by hemoglobin, unheated, and not over one day old.

To 41 c.c. of 2 per cent saline add 10 c.c. of 95 per cent alcohol and to each of the four tubes add 3.3 c.c. of this mixture.

Shake the tubes, heat in a water-bath at 60° C. for 30 minutes, let stand for 4 hours and read the degree of flocculation.

This is the technic described by Baum (Baum, F.: Am. Rev. Tub., 1924, x, No. 4, 449).

From a study of 300 cases including known tuberculosis, other diseases, and normal controls, Hardesty concludes that:

1. The reaction is nonspecific.

2. In general, tuberculous serum gives reactions the intensity of which is roughly proportioned to the severity of the process.

3. Normal serum and serum in diseases other than tuberculosis give negative or only weakly positive reactions.

4. The greatest value of the reaction is as an aid in the differentiation of active tuberculosis from other clinically similar conditions.

Cohen, M. B., Applebaum, H. S., and Hainsworth, E. L.: The Intracutaneous Salt Solution Test, Its Use as a Test of the Efficiency of the Circulation in the Extremities. Jour. Am. Med. Assn., May 29, 1926, lxxxvi, 1677.

When 0.2 c.c. of an 0.85 per cent salt solution is injected into the skin of a normal individual sixty minutes or more is required for its complete absorption. When edema is present the disappearance time is decreased in direct ratio to the degree of edema, disappearance in extreme cases being so rapid as to prevent the production of a wheal. These observations apply to all edema, regardless of its underlying cause, whether cardiac or renal.

This test, devised by McClure and Aldrich (Jour. Am. Med. Assn., May 3, 1924, lxxxii, 1425) is explained as due to the increased affinity of the tissues for water in edema leading to its rapid absorption from the most available source, in this instance the wheal produced by the injection.

During disease processes edema occurs whenever the water holding power of tissue colloids is increased and such increase can be caused by localized acidosis resulting from the accumulation in the tissues of acids, amines, or similar substances.

Localized acidosis can be produced by obstruction of the venous return and patients with varicose veins in whom the degree and duration of stasis can be regulated by posture afforded the authors an opportunity to study the effect of stasis on the salt solution disappearance time.

The following conclusions are formulated as a result of the study:

1. The intradermal salt solution test is a rapid and accurate measure of the tissue affinity of water.

2. The normal disappearance time is sixty minutes or more.

3. In all conditions associated with localized anoxemia the disappearance time is decreased.

4. In cardiac disease with decompensation the disappearance time is always twenty minutes or less in the edematous areas. If there is no decompensation, normal values are obtained.

5. In cases with peripheral arterial disease the disappearance time is always decreased in areas showing clinical involvement.

In 35 cases readings below ten minutes were found only in tissues immediately above gangrenous areas. Readings between ten and twenty-five minutes are strongly suggestive of developing gangrene.

Gilbert, R., and Humphrys, E. M.: The Use of Potassium Tellurite in Differential Media. Jour. Bact., February, 1926, xi, No. 2, 141.

A study of the differential value of tellurite medium in the isolation of B. diphtheriae. The method of preparing the medium is as follows:

Beef infusion agar 1.5 per cent	1 liter
Horse-serum (sterile)	5 per cent or 50 c.c. per liter
Glucose C. P., 20 per cent solution (sterile)	1 per cent or 10 c.c. per liter
Potassium tellurite C. P., 1 per cent solution (0.58 per cent on test)	1 per cent or 10 c.c. per liter
	1:34,000 Te, (1-17,000 K_2TeO_3)

Procedure.—Melt the agar and cool to 50° C. Add the serum glucose and tellurite solutions, all previously warmed to 50° C. Mix well and insert a sterile siphon. Dispense in Petri dishes. Incubate the plates and examine for contaminations before use.

The solution of potassium tellurite is prepared as follows:

Potassium tellurite C. P.	0.25 gm.
Distilled water	25.0 c.c.

Procedure.—Weight 250 mg. K_2TeO_3 on a chemical balance. Grind to a very fine powder in a small mortar and add about 10 c.c. of the water. Mix well, allow the undissolved portion to settle and pour off the supernatant fluid. Add more of the water to the residue, grind again and combine the two portions. Rinse the mortar with the remainder of the water.

The solution is then passed through a paper filter and then through the Mandler to sterilize it.

Note.—It is difficult to get this compound into solution and even after thorough grinding in the mortar, a small amount of fine suspension is visible. Heating gently to about 55° C., aids the action somewhat.

In this formula the tellurium content approximates 1:17,000.

Colonies of B. diphtheriae are white, or white around the periphery, with gray centers, the depth of color increasing up to 40 to 48 hours' incubation. Staphylococcus colonies are black. Colonies of B. hofmani resemble those of the diphtheria bacillus but have a heavier periphery.

Streptococcus colonies are white but smaller than those of B. diphtheriae.

The stock tellurite solution is, apparently, stable for at least twenty days.

From a study of various stock cultures it appears that certain gram-positive cocci, some of the higher bacteria, yeasts, and molds are only slightly inhibited in this medium, while many of the motile bacilli (typhoid group) and a number of anaerobes are markedly inhibited.

Atkins, W. R. G., and Pantin, C. F. A.: A Buffer Mixture for the Alkaline Range of Hydrogen-Ion Concentration Determinations. Brit. Biochem. Jour., 1926, xx, 102.

The following solutions are recommended for use from P_H 7.8-10.8, though they cover a slightly more extended range: *a.* 0.2 M. boric acid made 0.2 M. with respect to potassium chloride also, as recommended by Clark (1922). One liter should contain 12.4048 gm. boric acid (taking the atomic weight of boron as 11.0, not 10.9 the more recent value) and

14.912 gm. potassium chloride. *b*. 0.2 M. sodium carbonate 21.2000 gm. per liter, prepared anhydrous from the bicarbonate as is usual in alkalimetry. To make the mixtures for use the quantities of *a* and *b* shown in the table are mixed and diluted with 100 c.c. water.

The first three columns of the table record the P_H values found for various mixtures of the two solutions in multiples of 5 c.c. The last three columns show the volumes of the two solutions to be mixed to give P_H values differing by 0.2; the volumes were read off from a graph, which has been omitted to economize space. These buffer solutions keep well if stored in waxed bottles tightly stoppered.

WATER 100 C.C. AND BORIC SOLUTION	CARBONATE SOLUTION		BORIC SOLUTION	CARBONATE SOLUTION	
c.c.	c.c.	P_H at 16°	c.c	c.c	P_H at 16°
95	5	7.44	93.8	6.2	7.6
90	10	7.93	91.7	8.3	7.8
80	20	8.43	88.8	11.2	8.0
70	30	8.78	85.0	15.0	8.2
60	40	9.09	80.7	19.3	8.4
50	50	9.39	75.7	24.3	8.6
45	55	9.53	69.5	30.5	8.8
40	60	9.69	63.0	37.0	9.0
30	70	9.98	56.4	43.6	9.2
20	80	10.25	49.7	50.3	9.4
10	90	10.59	42.9	57.1	9.6
5	95	10.85	36.0	64.0	9.8
—	—	—	29.1	70.9	10.0
—	—	—	22.1	77.9	10.2
—	—	—	15.4	84.6	10.4
—	—	—	9.8	90.2	10.6
—	—	—	5.7	94.3	10.8
—	—	—	3.5	96.5	11.0

Jettmar, H. M.: Notes on the Viability of Plague Bacilli in Stained Smears. Nat. Med. Jour. China, February, 1926, xii, No. 1, 1.

The following conclusions are presented:

1. Intensive fixation by passing through the flame does not kill the bacilli in thin smears from a plague bubo.

2. Short fixation by alcohol after passing through the flame does not kill the bacteria with certainty. It seems that alcohol when used on a smear without previously passing it through the flame is more effective in this respect. However, no general rules can be drawn from these experiments, as they are too few for that purpose.

3. Preparations stained with diluted carbolfuchsin may still contain living plague bacilli even when treated previously with alcohol for a short time. It has to be supposed that a few bacilli managed somehow to escape the staining, though the slide was overstained. Vital staining is not likely to have taken place. It is remarkable that the whole procedure has done no damage to the surviving plague bacilli, as may be concluded from the short duration of the illness and the typical findings of acute bubonic plague in the guinea pig.

4. By staining with concentrated alkaline methylene-blue the bacilli seemed to be killed. The negative results obtained in some guinea pigs supports this contention. It may be supposed that this stain is so active because of the incomplete solution of its elements.

5. The wash from stained plague smears may contain plague bacilli. It has therefore to be disinfected, as well as the filter paper that has been used for drying the stained preparations. The bacilli seemed to be somewhat less virulent. As the wash was partly collected from alkaline methylene-blue smears also, and showed a blue color, this stain perhaps had some effect on the virulence of the bacilli.

6. As some of the infected animals succumbed and others did not fall sick at all, it has to be supposed that only a few plague bacilli were alive in the stained smears. Even the simple drying at room temperature was sufficient to protect the cutaneously inoculated guinea pig against infection.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The Chemical Analysis of Foods**

THE chemistry and chemical analysis of food is a subject which, within recent years, has undergone an extensive revision and expansion.

In this volume the elements of food analysis are presented in a very practical way and particularly for those who have not had special training or extensive experience in this field. The methods given are clearly described and have been well tried.

The scope of the volume is indicated by the following summary of its sections:

Sugars and sugar products (46 pages); starches and cereals (41 pages); baking powder and egg substitutes (11 pages); fruits and vegetables (25 pages); tea, coffee, and chocolate (26 pages); mustard, pepper, and spices (26 pages); wines, spirits, beer and vinegar (34 pages); flesh foods, meat extractives, and gelatin (18 pages); milk, and milk products (40 pages); butter, margerine, and cheese (22 pages); lards and oils (19 pages).

The book deserves a cordial reception from those to whom it is addressed.

A Manual of the Parasitic Protozoa of Man†

EPIDEMIOLOGY, preventive medicine, and even the practice of medicine in general have been very profoundly influenced by the march of progress.

Diseases hitherto confined to certain geographical localities are now to be encountered in far-flung places, carried there by modern means of transportation; others, hitherto considered peculiar to certain races, have been encountered in strange places as an aftermath of the unparalleled mixture of peoples during the World War. Especially is this the case in diseases due to protozoa, parasites responsible for some of the most important and serious diseases of man.

Colonel Craig's manual is not primarily a zoological treatise but is intended for the information and use of health officers and practitioners.

In it are presented in a clear, readable, and authoritative manner all the facts of real importance known about the parasites described.

The work is very complete and amply illustrated. Each organism is considered under the following headings: Synonyms, History and Nomenclature, Morphology (living and stained), Resistance, Habitat, Species Found in Lower Animals, Cultivation, Life History, Method of Reproduction, Geographical Distribution, Incidence of Infection, Method of Transmission, Experimental Infection, Relation to Disease, Pathology, Prophylaxis, and Diagnosis.

Under diagnosis all the laboratory methods of value are fully described and a technical appendix contains the most useful cultural and staining methods.

The book forms the most complete, informative, and satisfactory reference on protozoa which has yet been produced for the use of the practitioner, health officer, and laboratory worker, and it seems destined to become a standard in this field. It is attractively printed and deserves a wide circulation.

*The Chemical Analysis of Foods. By H. E. Cox, Public Analyst for The Metropolitan Borough of Hampstead. Cloth. Pp. 323, 38 illustrations. Price $5.00. P. Blakiston's Son and Co., Philadelphia.

†A Manual of The Parasitic Protozoa of Man. By Chas. F. Craig, Lieutenant Colonel, Medical Corps, U.S.A. Cloth. Pp. 569. 95 illustrations. Price $5.00. J. B. Lippincott Co., Philadelphia and London.

*Clinical Laboratory Methods**

THE second edition of this useful manual will be welcomed by physicians and laboratory workers.

The revision has been extensive and thorough. The chapter on blood has been extended and subdivided into five chapters corresponding to the method of approach in the clinical laboratory.

The section concerning the Wassermann reaction has been rewritten and greatly extended. Kolmer's qualitative and quantitative methods have been included; under the latter, however, the older and more cumbersome serum quantities are given rather than the later quantities incorporated in the method shortly after its publication and which greatly facilitate its performance.

New methods have also been added to the section on blood chemistry and, in fact, useful additions will be encountered throughout the entire book.

Particular care has been taken to make the index comprehensive and to include numerous cross references in both the index and the text.

The illustrations are good, the text well and clearly written, and the book in general in keeping with the standard of the publishers.

It will be found a useful and valuable addition to the library of any laboratory or physician.

Insanity and Law†

AS STATED in the preface of this book, there is a marked divergence in the point of view of the physician and the law leading to markedly conflicting opinions in relation to mental health, the physician being concerned primarily with the welfare of the individual, the law paying more attention to the effects of individual inadequacy on the community.

Much of the conflict thus produced depends not upon any fundamental conflict between medicine and law but upon an insufficiency of mutual understanding towards the better development of which this book is directed.

In this book an endeavor is made to state in simple language psychiatric facts which may be regarded as established, to present the legal aspects of mental disorder, and to outline the situation that now obtains both from the medical and legal point of view "with the hope of bringing about a better understanding of the actual basis from which must start any practical effort to improve the relations between insanity and law. The requirements for this improvement are two-fold: the physician needs a better knowledge of legal practice and ideals, and the lawyer needs fuller information about mental diseases," needs which this book attempts to supply.

For this task the authors are qualified by extensive classroom, courtroom and practical experience.

Part I, comprising 198 pages, is devoted to a consideration of mental disorders. A description is first given of certain types of reaction which may be encountered in many different diseases (delirium, hallucinosis, confusional states, dementia, etc.), followed by some account of the principal types of mental disease.

The remainder of the book—Part II—is devoted to the legal aspects of insanity and discusses: the legal definition of insanity; the determination of insanity; guardianship; insanity and contracts; insanity and marriage; insanity in relation to tort; insanity and criminal responsibility; insanity and testamentary incapacity; miscellaneous legal aspects of insanity; the physician in court; and some suggestions for reforms in procedure.

A Manual of Clinical Laboratory Methods. By Clyde L. Cummer, Associate Professor of Pathology, Western Reserve University. Second Edition. Cloth. Pp. 547. 169 engravings, and 12 plates. Price $6.50. Lea and Febiger, Philadelphia.

†Insanity and Law: A Treatise On Forensic Psychiatry. By H. D. Singer, M.D., Professor of Psychiatry, University of Illinois, and W. O. Krohn, M.D., formerly Head of the Department of Psychology, University of Illinois. Leatherette, $6.00. P. Blakiston's Son and Co., Philadelphia. 437 pages.

A glossary of terms is appended for the elucidation of the various technical—especially legal—terms necessarily employed.

The style is pleasing and as clear and readable as the complexities of the subject permit. A rather extensive experience in the courtroom and a consequent familiarity with—as they appear to the uninitiated—the ponderous pomposities of legal phraseology, lend, at times, a legal flavor to the presentation which seems to necessitate a reference to all judges as "learned" and all Courts as capitalized and partially deified beings of almost incredible wisdom albeit the irreverent might at times find some justification for picturing them as draped, not with the "majesty of the law," but with the absurdities of unending, tortuous, and obfuscating verbiage.

"The legal aspects of the relations of insanity to the various phases of business and social life have been set forth by quoting and discussing the laws; methods of procedure, and the important decisions of state and federal courts, because familiarity with these decisions will not only enable the physicians better to advise and assist the courts, but will also give a better understanding of the reasons that underlie the various technicalities."

This is a most interesting and readable section and well repays perusal.

There is much discussion of crime and the criminal these days and even the "learned Bar" has taken cognizance of what the untutored lay mind has long suspected: that the legal procedure of the present day is a ponderous structure some of the supports of which are so archaic as to give a somewhat Pisa-like tendency to lean more toward the safe guarding of the criminal than toward the protection of the community.

It is somewhat difficult for the unlegal mind—if it may be so termed—to avoid the impression that more attention is paid to what was said by the learned Court in the case of Woofenpoof vs. the people in 1721, than to whether or not the defendant actually robbed the bank or murdered his aunt; that too many verdicts are nullified on the tenuous grounds of formal technicalities, misplaced commas and similar absurdities, or to understand why it often appears that legal defense procedures are apparently synonymous with attempts to evade the law and its provisions by dexterous and sinuous counter-procedures if not by more questionable means.

The perusal of this book will be of value to both the physician and the lawyer. It will not make of any physician a psychiatric expert but it will inform him as to the necessary array and extent of information required by such a position, warn him against rash ventures into such fields, and render him better able to avoid embarrassing situations if forced by circumstances to take the stand either as an ordinary or expert witness.

It will not make of the lawyer a psychiatrist, but furnishes in compact form a mine of information and if honestly read and thoroughly considered should emphasize the necessity of something more than a desire to escape punishment as a reasonable ground for a claim of insanity.

The hypothetical question is discussed at length and its inherent possibilities for misleading the jury noted and the opportunity it affords for legal trickery touched upon, though with all due courtesy to the legal profession and its somewhat tender sensibilities. The extreme latitude allowed in the cross-examination of witnesses—which not infrequently resembles bullying—is mentioned in non-committal fashion.

A more expanded consideration of needed reforms in procedure seems possible—the experience of the authors could well be brought to bear upon this point. The Bar has it now under consideration and in view of the apparently constitutional inability of the legal mind to divest itself of cumbersome and ponderous technicalities, the medical expert could surely contribute something, providing he *is* a clear-thinking and qualified *expert* and not a professional witness.

As psychiatrists the authors take the orthodox view of the criminal as one requiring treatment rather than corrective measures tending toward the protection of the community of which he is an individual, though this aspect is but briefly touched upon.

In the last analysis this book is a valuable contribution and though most useful to the lawyer and the medico-legal expert, it can be read with profit by both professions at large.

*Medicine: An Historical Outline**

HE WOULD be brave, indeed, who would suggest additions to the overcrowded medical curriculum, but to advocate some discussion, however brief, of the history of medicine is a stand for which a strong defense may be made.

The modern graduate is forced to cover a vast amount of territory unexplored by, and perhaps, even unknown to, his predecessors of a few decades ago and yet it may be regretted that, too often, his knowledge and his reading are apt to be restricted to the technicalities of his profession. It is necessary, of course, and an advantage to assay the difficult task of keeping up with medical literature; it is equally advantageous to broaden the field of one's reading—and seldom can this be done with more interest or greater profit than by excursions into medical history.

In the introduction to the "Arabian Nights" occurs the following: "The lives of former generations are a lesson to posterity; that a man may review the remarkable events which have happened to others and be admonished; and may consider the history of peoples of preceding ages and be restrained."

In this book are presented the substance of eight lectures in which an outline of medical history from primitive times to the nineteenth century is presented to the students of the Washington University Medical School.

It is obvious that in this limited space no more than an outline can be presented—and no more is attempted. Medical history is a study in transitions and these transitions are vividly as well as attractively sketched. The volume is one which will stand rereading.

The primary purpose is to arouse interest and to stimulate the appetite.

"Books are for nothing but to inspire," says Emerson, and Goethe tells us that "the best we derive from history is the enthusiasm it excites in us."

Dr. Seelig's subject is interesting in itself and this interest is intensified by his style and manner of presentation.

"If in any vacant vague time you are in a strait as to choice of reading," Carlyle once said, "a very good indication * * * * is toward some book you have a great curiosity about." Dr. Seelig's outline is well calculated to stimulate and arouse curiosity about the worthies of olden days of whom he speaks entertainingly and so, indirectly, to lead to further reading—and "reading maketh a full man" just as "studies pass into and influence manners."

Francis Bacon tells us that "some books are to be tasted, others to be swallowed, and some to be chewed and digested," that is, read with diligence and attention.

It is difficult to imagine the physician without interest in the history of his profession. If such there be, Dr. Seelig's outline will serve, without fail, to whet his appetite and introduce him to an interesting, absorbing, and profitable relaxation for his odd moments and leisure hours.

The volume is well printed though not quite attaining the motto of the publishers—"sans tache."

A more attractive paper might also have been used.

An introduction by Dr. Garrison and numerous plates of outstanding medical celebrities complete a very interesting, well-written, and readable book.

The Nematode Parasites of Vertebrates†

A VERY comprehensive and copiously illustrated determinative manual which should be a useful addition to the reference library of the pathologist and parasitologist.

*Medicine: An Historical Outline, by M. J. Seelig, M.D., Professor of Clinical Surgery, Washington University School of Medicine, Cloth, $2.50. Williams and Wilkins Co., Baltimore, Md. Pp. 207, 27 illustrations.

†The Nematode Parasites of Vertebrates, W. Yorke, M.D., Professor of Parasitology, University of Liverpool, and P. A. Maplestone, M.D. with an introduction by G. W. Stiles, Professor of Zoology U. S. Public Health Service, Cloth, $9.00. P. Blakiston's Son and Co., 536 pages, 307 illustrations.

*A Textbook of Pathology**

THE third edition of this well-known work needs no introduction and but little comment. Constructed upon the conception that all pathologic disturbances are the result of some form of injury, or the immediate or remote reactions of the body to injury, the book is planned to discuss disease as far as possible upon the basis of etiology.

This conception is carried out quite logically except, of course, in the consideration of tumors, the etiology of which, as yet, remains unknown.

The style is clear and most interesting and the typography pleasing.

A constant effort has been made to speak of the disturbances of function and of chemical interchange in the course of disease, and even to describe symptoms. If, as the author states, this makes the book seem a treatise on clinical medicine, it is only because pathology and clinical medicine are the same thing viewed from slightly different angles.

While not intended as a book of reference, the volume is one of equal interest to clinicians and pathologists and one which should be well read by both. It can be highly commended.

Middle Age and Old Age†

A DELIGHTFULLY written book on longevity and how to acquire it which contains, as is so often the case in writings on this subject, rather frequent statements supposedly of proved facts which the reviewer at times hesitates to accept unquestionably as such. The writer's chief premise is against faulty dietary habits, and many of the infirmities which beset man past middle life are attributed primarily to intestinal toxemia. The endocrine organs come in for their full share of discussion but are on the whole treated conservatively. Rejuvenation operations are recognized but the author feels that the Steinach operation has not been a success. He is rather more optimistic concerning the Voronoff gland transplantation.

As we have stated, exception might be taken to some of the stands taken by the author. Thus he objects to saccharose in the diet on the grounds that since glucose alone is utilized, energy is wasted and perhaps the system is to an extent poisoned by the necessity for breaking down and absorbing this foreign sugar. In replying we would raise the question, "Why not on the same grounds prohibit all starches?"

Again he emphasizes that a high carbohydrate diet changes the colon bacillus from a putrefactive organism into a friendly fermentative organism. We would raise the question whether it is the same germ with which we are dealing in the two instances.

He recommends occasional fasting of three or four days or longer as being highly beneficial. We wonder whether acidosis is a sign of health.

The author looks upon the irritation of the adrenal glands as the determining cause of chronic interstitial nephritis. This has scarcely been generally accepted.

However, aside from dogmatic generalization which must be accepted with reservation, the volume contains much food for thought, and scattered throughout one runs upon unexpected aphorisms that it is a joy to read.

Pygmalion or the Doctor of the Future‡

THIS small volume appears as one of the "today and tomorrow" series, a series of contributions by prominent writers describing present-day achievements in all phases of life with prophetic remarks on future developments. Dr. Wilson has written of the physician of the future. He provides ninety minutes of stimulating and enjoyable reading but scarcely covers the task assigned. His thesis is the true significance of symptoms. His ex-

*A Textbook of Pathology, by W. G. MacCallum. Cloth, $10.00. W. B. Saunders Co., Philadelphia, 1162 pages, 575 illustrations.

†Middle Age and Old Age. By Dr. Leonard Williams. Cloth. Price $3.25. Pp. 296. Oxford University Press, 1925.

‡Pygmalion or the Doctor of the Future. By R. M. Wilson, M.B., Ch.B. Cloth, price $1.00, pp. 67. E. P. Dutton & Company.

planation of their origin and significance is truly interesting and gives one cause for thought even though one concludes at the end that he has not proved his point and that the true explanation will ultimately be along different lines. Perhaps he sketches his theory too hurriedly, but we feel that he has not proved his point, and there arises within us the suspicion that in actual practice he must necessarily and not infrequently have recourse to the more orthodox therapeutic measures.

Nevertheless there is much obvious truth in many of the illustrative anecdotes and one cannot but read the volume without becoming a little better as a physician and more inclined to treat the patient as a living individual, attending not only to his body but also to his mind. This is the basis for the title of the book and will be appreciated by all who know the story of Pygmalion. "I look forward to the time when the practice of medicine will include within its scope every influence of known potency over the human spirit and when the practitioner, like Pygmalion, will look on his work and see, not disease and death, but glowing lineaments of life." This characterization of the doctor of the future will, we hope, be true, but the author's portrayal of future advances in medicine as being dominantly along psychologic lines is surely incomplete.

*Lactobacillus Acidophilus**

SINCE the use of acidophilus bacillus in the treatment of intestinal diseases, particularly constipation, has come into quite general use, and since the market has been flooded with preparations of questionable value, it is high time that an authoritative treatise on the subject should appear so that we may acquire a more thorough knowledge of the rationale of such treatment.

The author starts in with an unusually critical survey of the literature, pointing out inaccuracies and erroneous deductions in the works of his predecessors, and errors resulting on incomplete experimental observations. The reviewer's natural reaction is toward critical judgment of the present author and a search for the weak points in his argument. At the end of the book we confess that they are surprisingly few. The work is comprehensive.

While Kopeloff is a firm believer in acidophilus therapy when properly applied, he points out convincingly that usually it is not so applied. Scarcely any of the commercial acidophilus preparations are of true reliability. The only preparation containing sufficient viable organisms for successful results are the acidophilus milk preparations. Even here the odds are against the commercial products, for in his own laboratory he finds that after preparation of the milk the number of the viable organisms rapidly diminishes after the third or fourth day. Very rarely can one obtain a commercial preparation of milk less than four days old.

He shows that the action of lactobacillus acidophilus is not a physical phenomenon, since sterilization of the milk destroys its remedial potency. Nor is it strictly chemical, since patients receiving pasteurized acidophilus milk were not relieved of constipation and the administration of acidophilus milk which had been filtered through porcelain was without effect. Sterilized acidophilus milk to which lactic acid had been added was not successful. The data indicate that this form of therapy is essentially bacteriologic rather than physical in nature.

A Handbook for Senior Nurses and Midwives†

A THOROUGHLY comprehensive textbook on medicine and surgery and obstetrics for the use of nurses. To one who endeavors to include between the covers of one volume such a wide expanse of medical territory, the problem at once arises as to what shall be incorporated and what omitted. The author has indeed done well in omitting all rare and

*Lactobacillus Acidophilus. By Nicholas Kopeloff, Ph.D. Associate in Bacteriology, Psychiatric Institute, Ward's Island, N. Y. Cloth. Price $5.00, pp. 221. Williams & Wilkins, Baltimore, Md., 1926.

†A Handbook for Senior Nurses and Midwives. By J. K. Watson, M.D., (Edin.) Capt. R.A.M.C. Cloth. Price $4.00. Pp. 554. Oxford University Press, 1926.

unusual conditions, but we feel that he has failed to include certain diseases which should be discussed and has perhaps gone into unnecessary detail with others. The medical section of the book is the smallest, the surgical the largest. In the former we should like to see some mention of those most common of medical conditions, nephritis, hypertension, arteriosclerosis and cardiac disease. We feel that for the nurse a knowledge of the treatment of nephritis and diabetes is more important than the treatment of gonorrhea in the male.

Likewise in the obstetric and gynecologic sections we should like to see more details of the relationship between pregnancy and acute and chronic disease in the mother.

Aside from this difference of opinion as to what subjects should be included in such a text, the volume appears to fill excellently the function for which it was intended. The description of operations is sufficiently detailed so that the nurse may know the rationale thereof.

We are glad to note that the author recommends preliminary medical treatment in gastric and duodenal ulcer before resorting to surgery. His discussion of the use and indications and counterindications for pituitrin in obstetric practice is excellent. In the orthopedic section we find good illustrated descriptions of reconstructive exercises such as are usually applied by the nurse. A most important chapter is on constipation. Far too many patients nowadays develop the cathartic habit while in the charge of a nurse. The directions for the nursing care of acute conditions such as appendicitis before and after operation is presented in a better manner than usual.

The section on pediatrics covers particularly appropriate subjects for the information of the nurse. We hope that in the next edition more space will be available for a discussion of nuritional disorders in infancy and childhood and newer methods for their treatment and prevention.

*Comparative Physiology**

THE science of comparative anatomy and embryology has perhaps more than any one method of investigation enabled us to understand clearly the process of fetal development.

Much of our present knowledge of human and mammalian physiology is based on studies of comparative physiology. Indeed, we feel secure in the statement that in spite of tremendous recent advances resultant on increasing knowledge of such sciences as biochemistry, much of our future advancement in physiology and functional pathology will still be dependent upon studies in comparative physiology.

Take, for example, the function of the kidney. Does the glomerulus merely serve as a filter allowing the blood crystaloids to pass through, separated from the blood proteins? Is the secretion of the urine through the glomeruli dependent merely upon the balance between the vascular blood pressure and the opposing osmotic pressure? Does the tubular epithelium secrete actively or does it resorb? While these questions have not as yet been conclusively answered much light has been shed upon them by the fact that in other animals in which the anatomic arrangement is different from that of mammals, we may study individual functions. Thus in the frog the glomeruli receive vessels from the aorta while the tubules are supplied with blood vessels from the portal system. One blood supply may be cut off with the other left intact. This has given us the definite information that with the supply to the glomeruli cut off, the kidneys do not secrete urine. Again, certain fishes have such a low blood pressure in the aorta that in studies of the physiology of urinary secretion we may eliminate this factor to a certain extent. It seems unlikely that there is ever a blood pressure in the renal vessels of the fish sufficient to overcome the osmotic pressure of the blood colloids. If this is actually the case, filtration clearly plays no part in the renal function of fishes. True, the last word has not been said in renal function but without comparative physiology, our knowledge would be considerably retarded.

The *magnum opus* on this subject up to 1910 has been Winterstein's *Vergleichende Physiologie*. This is of encyclopedic proportions and should be known to all who are in-

*Comparative Physiology. By Lancelot T. Hogben, M.A., (Cantab), D.Sc. (Lond) Assistant Professor in Zoology, McGill University. Cloth. Pp. 219. The Macmillan Company, 1926.

terested in the subject. The present contribution, while covering the realm of general physiology, aims particularly to bring out the works of importance which have appeared since 1910. The author divides his discussion into four sections. The first to manifestations of vital activity such as muscular contraction, capillary activity, ameboid reactions, color response and secretion; the second to metabolism or the sources of vital energy, including respiration, nutrition and circulation of body fluids; the third to coordination, the integration of vital activity, including endocrine coordination, the mechanism of nervous conduction and the analysis of behaviour in animals; the fourth to reproduction, the building up of a new animate unit. Here the author discusses comparative knowledge obtained on the fertilization of the egg, inheritance and the physiology of development. Taken as a whole, the volume is quite complete in its outlook.

Aside from its scientific interest to students of zoology or physiology the work is delightful general reading in that it explains curious everyday phenomena such as the change of color of the lizard or toad, the mechanism of movement of the ameba, respiration in fishes, the manufacture of poison in salivary glands, of strong sulphuric acid in salivary glands, the storage of oxygen in gas bags in certain fishes, such as the "puffer fish" or balloon fish, and the secretion of luminous material.

*Kidney Diseases**

A MANUAL for victims of nephritis and hypertension written along lines analogous to those followed by Joslin in his famous manual for diabetics. The basis of treatment outlined by the author is primarily salt restriction, especially in hypertension. At the same time proper emphasis is placed on nitrogenous restrictions and other therapeutic measures. This being in essence a manual for the use of patients and physicians, the author does not include any large volume of statistical or experimental evidence substantiating his contention that an error of salt metabolism is a basic factor in the etiology in hypertension, but he rather goes ahead placidly assuming that this has been conclusively demonstrated and outlines treatment on this basis. Dr. Allen is convinced that in his hands radical salt restriction has produced better results than any other single method. In his introduction he writes: "If the proposals are valid, they will revolutionize the treatment of high blood pressure and effect a corresponding saving of health and life. If they are mistaken, the writer persists in the mistake after six years of careful study and is prepared to bear the consequences."

The author's conception of the etiology of diseases of the kidneys, blood vessels and pancreas as a tissue sensitization consequent on a primary injury, usually a severe acute infection is most logical. Even though perfect recovery appears to follow this infection, the kidneys and blood vessels are left as a *locus minoris resistentiae* for all subsequent infections and intoxications. At the same time the damaged organs probably become abnormally susceptible to functional wear and tear. In this way we can plausibly explain the gradual onset and progressiveness in renal-vascular disorders months or years after the primary infectious cause has disappeared and we have justification for the *prophylactic* use of dietary and other restrictive measures.

The author devotes chapters to diet in general, to protein restriction, to salt restriction, to the various laboratory procedures, and to useful recipes.

In discussing the laboratory examinations including functional studies, Dr. Allen does not attempt to describe all of those tests even that are in rather general use, but much information is to be gained from a reading of those that he does describe.

While the author does not claim that it is essential in these conditions always to weigh the food, he provides simple instructions for accurately weighed and controlled diets. His table of the salt content of foods is the most complete we have seen. He also provides general food tables of acid-forming and base-forming foods.

*Treatment of Kidney Diseases and High Blood Pressure. By Frederick M. Allen, M.D. Part I. Practical Manual for Physicians and Patients. Cloth. Pp. 206. The Physiatric Institute. Morristown, N. J.

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EDITORIALS

The Functional Pathology of Nephritis

NEARLY a century ago Richard Bright published his description of a group of cases illustrative of that condition which carries his name. He recognized the occurrence simultaneously of abnormal constituents such as albumin in the urine, and clearly abnormal kidneys at necropsy. He found that the pathologic changes in these organs was not always the same and was unable from study of the urine during life, to prognosticate clearly the distribution of structural alterations. Ninety-nine years have elapsed and while we know far more concerning both the physiology and the pathology of the kidney, we must still confess that even today we usually cannot conclusively localize the renal damage even after having had an opportunity to apply all of the present methods of uranalytic and blood chemical studies.

In the days when the structural pathologist held dominion, the problem appeared solved and the classification of the chronic nephritides was clearly divided into groups such as parenchymatous, interstitial and glomeruloneph-

ritis. Weigert then proceeded to demonstrate that chronic parenchymatous lesions are nearly always associated with interstitial growths and interpreted the connective tissue proliferation of interstitial nephritis as an attempt to fill in the defect caused by the destruction of parenchyma. He considered this essentially a replacement fibrosis. Interstitial nephritis then went into the discard.

The classification seemed then to be entirely unambiguous, the large white kidney being associated chiefly with parenchymatous nephritis, the secondary contracted kidney being the end stage of a chronic glomerulonephritis and the primary contracted or red granular kidney with its fibrous tissue, enclosing atrophic tubules and obliterated glomeruli and alternating with active parenchyma, being the terminal picture of a renal arteriosclerosis.

But the unexpected surprises at necropsy still came too frequently. From a study of the specific gravity of the urine and the relative proportions of albumin and casts and other constituents, anatomic diagnoses were made which were not borne out after death.

Today studies of renal function enable us to ascertain with reasonable certainty the degree of parenchymatous destruction but we are not yet in a position to designate the exact location of the lesion in the individual case. The more recent method of approach to such a localization has been through a minute study of the physiology and pathologic physiology of the organ rather than along anatomic lines. This has carried us quite far but the situation at present is rather anomalous since in certain types of nephritis the "functional" localization of damage is distinctly different from what would appear to have been proved on "anatomic" grounds. As yet no completely satisfactory reconciliation of this divergence has been reached. Mayrs in discussing parenchymatous nephritis writes as follows: "Hydremic nephritis may be regarded as a disease mainly involving filtration; the glomeruli having become permeable to protein, while the function of the tubule cells is less affected. This view seems inconsistent with the microscopic changes found in the kidney. The glomeruli may appear little altered, while the tubule epithelium shows cloudy swelling, fatty degeneration, and often more serious impairment."

Bowman (1842) postulated the secretion of water through the glomeruli, which passing down the tubules washed away the urinary excretory products as they were secreted by the tubular epithelium. Ludwig, two years later, advanced a theory of the glomerular secretion of a protein-free blood filtrate, its crystalloid constituents in the same concentration as in the latter, with the subsequent reabsorption of water and some of the solid constituents, through the tubules. Thirty years after Ludwig's publication, Heidenhein believed that he had disproved Ludwig's theory by the fact that certain dyes injected into the circulation appeared to be eliminated by the tubule, the fact that hypothetical increase of pressure in the glomerular vessels following partial obstruction of the renal vein did not increase the output of urine, and by the argument that the estimated volume of blood flow through the renal vessels per day was insufficient to account for the amount of urea excreted, assuming that it was all excreted purely by glomerular filtration,—that the amount of filtration must be incredibly large compared with the volume of blood flow.

Heidenhein hypothecated the active secretion of water and chlorides through the glomeruli, to which was added the urea, uric acid, dyes, etc., which were secreted as the urine passed through the tubules.

In 1917 Cushny formulated the so-called modern theory in which he conceived that a protein free filtrate passes through the glomerulus and that this filtrate contains urea, uric acid, sugar, amino acids and chlorides. In the tubules most of the water and chlorides are reabsorbed together with all of the sugar and the amino acids. The urea passes on in the urine. The value of Cushny's theory lies in the fact that it corresponds more nearly than any of the others to our existing knowledge and it serves to explain more satisfactorily the pathologic changes observed. None would say that it is in its entirety the true explanation of the secretion of urine, but it is surely the best we have.

The experiments of Richards, Wern and their collaborators have clarified greatly our understanding of glomerular function. Prior to their work all experiments on the relationship between blood pressure and blood flow through the kidneys and diuresis had at least two variables. They were able to test the validity of the filtration hypothesis by keeping all recognizable conditions constant with the single exception of the pressure of blood flowing through the vessels of the kidney. They found that under conditions which completely prevented alterations in the rate of blood flow through the kidneys any considerable increase in pressure was associated with a parallel increase in urinary output. This simple fact of mechanics obviously served to corroborate the filtration hypothesis.

They have further examined urine collected from the intracapsular space before it has passed into the tubules and find that this fluid contains no proteins, but does contain both sugar and chloride. Sugar is present in the glomerular filtrate when it is absent in urine collected from the bladder. Chloride is a normal constituent of bladder urine but in frogs which had been kept for a time in distilled water chloride disappeared entirely from the bladder urine and yet was still present in the glomerular filtrate. These facts present "incontestible proof of the reality of absorption within the tubule and provide the basis for further argumentative support of the view that the glomerular function is a physical rather than a vital or secretory one." On the other hand, a slightly greater concentration of urea and of chloride was found in glomerular urine than in the blood plasma. However, they believe that this slight increase can be explained on the filtration hypothesis and does not of necessity indicate a true secretion. The fact indicates, however, the necessity for a study of the manner in which capillary forces and vitality of the glomerular membrane may influence the filtration process. In this connection Mayrs points out that chloride increase is characteristic of a number of capillary exudations (aqueous humor, cerebrospinal fluid) and may be explained by the Donnan membrane equilibrium.

By carefully controlled experiments these workers have shown quite conclusively that constriction of the efferent arterioles leading from the glomeruli causes coincident increase in general blood pressure, diminution of volume of blood flow through the kidney, increase in size of the kidney,

with increase in the size of the individual glomeruli, and an increase in urinary output. Increase in blood pressure causes increased secretion of urine. This increase is effected through constriction of the efferent arterioles.

They have shown that the dilator effect of a constituent of the blood is exerted more markedly on the afferent vessels of the glomeruli than on the efferent, due probably to the fact that the blood in the two vessels is really quite a different fluid, being much higher in colloid concentration in the efferent arterioles. This dilator phenomenon would result in an increase in intraglomerular pressure, and a resulting diuresis.

On the other hand, the secretion of urine may be lessened by still greater vasconstriction such that the afferent vessels are also markedly constricted. Another mechanical factor which serves as a brake on the secretion of urine, is the elasticity of Bowman's capsule itself, preventing undue distention of the capillary tuft.

White and Schmitt, while not accepting without reservation all of the conclusions reached by Wern and Richards, have been able to substantiate their observations on the constitution of the protein-free plasma filtrate. They further show that sugar and chlorides are both reabsorbed in the proximal convoluted tubules. Instead of the frog they use necturus, an amphibian whose kidney they believe is better adapted to this form of micromanipulation. In this animal they can obtain fluid from any portion of the proximal convoluted tubule, and they find that sugar, while consistently present in the intracapsular fluid, has disappeared by the time the urine has reached halfway down the proximal convoluted tubule. Their experiments in demonstrating the site of absorption of chlorides are ingenious but will require confirmation.

So much for the physiology of the secretion of urine. The evidence would indicate that a protein-free filtrate passes mechanically through the glomerular capsule and that certain of the constituents, particularly sugar, chlorid and water, are reabsorbed, presumably through the agency of cellular activity, as they pass through the convoluted tubules. Whether the passage through the glomerulus is a matter of filtration or whether in addition there is some activity of the epithelial cells covering the tuft has not been conclusively decided, but the weight of evidence favors a simple mechanical filtration. Likewise, the possibility of coincident excretion through the tubules has not been absolutely refuted.

Mayrs attempts to correlate the symptoms of nephritis and to explain them in the light of what is known of the physiology of the kidney. He recognizes filtration and concentration as the two most important phases in the production of urine, and classifies nephritis in so far as possible into diseases affecting these two functions. For this purpose he adopts MacLean's division into "hydremic" and "azotemic" types as being roughly a distinction between filtration and loss of concentrating power. Acute nephritis with accompanying water retention and chronic parenchymatous nephritis with edema, exemplify the hydremic type, while chronic glomerular nephritis with retention of the nonprotein-nitrogen constituents is classed as azotemic. Frequently, particularly in the advanced stages, the two types are intermingled.

Chronic parenchymatous nephritis is characterized by an excessive albuminuria, edema, slight or considerable reduction in the volume of urine, retention of chlorides, with at the same time efficient excretion of nonthreshold bodies such as urea. In this condition Mayrs believes that the major pathologic change is in the glomeruli rather than in the tubules, the disease involving mainly the function of filtration. The glomeruli, while showing little physical alteration, have become permeable to the serum proteins as in orthostatic albuminuria. The fact that microscopically the glomeruli do not appear greatly diseased is according to the author not conclusive, for this is true likewise of capillaries involved in inflammatory changes elsewhere in the body.

He accepts Epstein's suggestion that a fall in the protein osmotic pressure of the blood plasma is the cause of edema in *hydremic* nephritis. He details the various arguments pro and con and gives confirmatory experimental evidence of his own. Formerly it was held that edema was caused by a hydremia from failure to eliminate water. This would indicate an increased amount of fluid in the blood but recent observers have found no increase in plasma volume, indeed sometimes an actual decrease.

The plasma proteins possess a not inconsiderable osmotic pressure. Starling found that normal plasma could hold water against a pressure of about thirty millimeters of mercury. This property of the plasma protein tends to prevent fluid being forced into the tissues by the force of capillary blood pressure. The loss of protein through the urine so lowers its concentration in the plasma that it no longer holds fluid in the vessels. Water then passes out into the tissues. Where the capillary pressure is high, fluid passes out into the tissues and where the pressure is low it is not reabsorbed as it should be because of the reduced protein osmotic pressure.

Mayrs has developed a relatively simple instrument for the determination of the blood protein osmotic pressure and finds that in so-called parenchymatous nephritis there is a very decided drop in this pressure. In these cases the pressure was not much more than a quarter of the normal, while at the same time the plasma had quantitatively more than half of its normal protein content. This would suggest a change in the character or proportions of the blood protein constituents. He finds that the determination of plasma osmotic pressure is of diagnostic value in determining the cause of edemas.

The reduced volume of urine in this form of nephritis might be due to impaired secretion through the glomerulus. This appears scarcely a tenable hypothesis if at the same time we presuppose a hyperpermeability to proteins. It may be due to diminished glomerular filtration resultant on a lessened blood flow through the inflamed kidney; or, more fluid may be reabsorbed through the tubules. Mayrs feels that probably loss of fluid from the blood into the tissues is compensated as far as possible by excessive reabsorption from the renal tubules.

The author does not look upon chloride retention as a factor of great importance in the causation of nephritic edema. There is usually some retention in this condition but no more than occurs in some other diseases without kidney damage and he has found equally high plasma chloride figures in

cardiac edema. Furthermore, chloride retention occurs in diseases not associated with edema.

Mayrs suggests that the supposed beneficial effects of calcium in lessening nephritic edema may be due to the action of this chemical in diminishing capillary permeability. In this case the diminished capillary permeability in the glomeruli promotes retention of plasma protein. He feels that this is more likely to be the true explanation than the more commonly accepted one that calcium alters the salt balance in the body.

While he attaches little importance to salt restriction and states that there is no evidence that moderate excess of chloride in the blood causes specific symptoms, he does state that the possibility of disturbances in osmotic equilibrium between the cells and fluids of the body appears to justify the exclusion of salt from the diet.

With regard to chronic glomerular or azotemic nephritis, the author, while recognizing that histologically greatest damage appears in the glomeruli, localizes the pathologic functional changes, mainly in the tubules. He points out that, while many of the glomeruli are badly diseased or even destroyed, others which are either normal or in relatively good condition, are carrying the burden. The presence of slight albuminuria indicates that there is at least a little functional damage to the glomeruli. This, provided we assume that protein cannot emanate from the tubules.

As the glomerular filtrate passes through the tubules water is absorbed, perhaps by osmosis, and chlorides are absorbed actively by the tubule cells. These cells are resistant to the absorption of nonthreshold bodies such as urea, uric acid and creatinine. When, however, they are damaged as in chronic glomerulo-nephritis their impermeability to these substances becomes lessened and, as water passes through them into the blood, the dissolved nitrogenous substances are not resisted as successfully and they pass back with the water and chlorides.

The cells offer a lessened resistance to diffusion through them of nonthreshold bodies. This resistance is to an extent selective in that cells become permeable to uric acid before they do to urea, and only in the later stages do they become markedly permeable to creatinine.

With this diffusion through the cells the blood uric acid, the blood urea and creatinine gradually increase. The greatest hope of keeping the level of the blood reasonably low consists in diuresis such as usually occurs in chronic glomerulo-nephritis. The promotion of diuresis with caffein or theobromin should theoretically be logical therapeusis since these drugs appear to act chiefly by increasing glomerular filtration. But it is doubtful whether they could alter an output of urine already considerable. Saline diuretics on the other hand act in the tubules as nonthreshold bodies and merely increase the amount of waste material to be eliminated.

Apparently the renal tubules never lose entirely their ability to actively absorb and concentrate chlorides, even in the later stages of chronic nephritis. The function, however, becomes impaired to an extent.

While Mayrs' proposed explanation of the pathologic functional processes is interesting, and, taken as a whole, presents a picture which can easily

be visualized, much remains to be explained and certain paradoxical situations such as the difference between the proposed functional localization and the known microscopic localization must be reconciled.

This review also emphasizes the unsatisfactory state of the present nomenclature. MacLean's recent division into "azotemic" and "hydremic" types becomes unacceptable if in the latter there is no actual increase in the fluid content of the blood. It would be better for the present at least perhaps to adhere to a nondeterminate classification, such as that recommended by Christian, using such terms as chronic nephritis, nephritis with or without hypertension, chronic nephritis with edema, etc. Not until we learn more of the modus operandi within the diseased organ can we venture to make a thoroughly satisfactory descriptive classification.

REFERENCES

Richards, A. N.: Nature and Mode of Regulation of Glomerular Filtration, Am. Jour. Sc., December, 1925, clxx, 781.

White, H. L., and Schmitt, F. O.: The Site of Reabsorption in the Kidney Tubule of Necturus, Am. Jour. Physiol., April, 1926, lxxvi, 483.

Mayrs, E. B.: The Functional Pathology of Nephritis, Quart. Jour. Med., April, 1926, xix, 273.

See also discussions of nephritis in the *Book Review Section* of this issue, under "Comparative Physiology" and "Kidney Disease."

—*W. T. V.*